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Why settle? A review of timely and premature brewing yeast flocculation

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Abstract

Why was the work done: Yeast flocculation is an important parameter in brewery fermentation, as beer quality is affected by the onset and intensity of cell aggregation. Malt induced premature yeast flocculation (PYF) is described as a rapid decline of yeast cells in suspension during fermentation even in the presence of sufficient nutrients. Though occurring only sporadically, premature yeast flocculation can represent a significant risk to brewery operations.
What are the main findings: This review considers the known and suspected mechanisms underlying both normal and abnormal flocculation. Particular effort is made to propose definitive methods for the detection of PYF and to summarise potential strategies to alleviate premature yeast flocculation, with emphasis on relevant approaches for industrial brewing.
Why is the work important: Recent research has provided new insights to elucidate the mechanisms involved in premature yeast flocculation, though a complete understanding has yet be reached. Given the association of PYF with poor field conditions during barley growth, it may be expected that climate change will contribute to an increase in the frequency or severity of PYF. This review is intended to serve as a resource for further research on the topic as well as development of practical prevention or mitigation strategies.

Keywords

Premature yeast flocculation, malt, barley, brewing, yeast, mitigation

Introduction

Timely and complete yeast flocculation and sedimentation is important for the efficient production of high quality beer. Perturbations to the process can result in beers that are difficult to clarify, or beers that suffer from incomplete fermentation or flavour defects. Premature yeast flocculation, referred to as 'PYF', is the focus of this review. This defect in flocculation results in an early reduction in the numbers of cells in suspension during fermentation. This typically occurs before all fermentable sugars have been consumed, producing a beer that is out of specification for the brand, or necessitating interventions such as extended fermentation time, or downstream blending (Speers 2016).

Despite the risks that PYF poses to brewers, the mechanisms involved remain unclear. This is understandable given that yeast flocculation - due to its complexity - is poorly understood. What is clear is that the PYF phenomenon is associated with malt of compromised quality (Kaur et al. 2012; Macintosh et al. 2014). Often, this is linked to contamination in the field with *Fusarium* spp. or other microbial pathogens. As contamination is related to weather conditions in the growing season, and weather patterns are affected by climate change, it may be expected that PYF will be observed more frequently in the future.

This review highlights recent developments in the field and provides a resource for future research on PYF. Included is an overview of standard flocculation, the mechanisms involved, and a description of PYF together with the possible underlying mechanisms and causes. Also included is a summary of methods used to assess PYF, and an account of how it may be mitigated at a practical level in the brewing industry.

Yeast flocculation

Yeast flocculation is an asexual, reversible, and calcium dependent process of cell aggregation in which homotypic cells adhere to each other to form clumps and subsequently settle out of the medium in which they are suspended (Stratford 1989a, 1992; Stewart 2018). The brewing industry benefits from this process, which represents a simple and cost effective way to separate yeast cells from beer toward the end of fermentation. Further, this behaviour enables consecutive rounds of fermentation, with cropping, storage and repitching (Verstrepen et al. 2003).

How and when yeast flocculates is critical for beer quality as the number of cells in suspension during the course of primary fermentation has a direct influence on the rate and degree of fermentation, beer flavour, duration of the maturation phase, and ease of beer clarification (Axcell et al. 2000; Speers 2016). Even though flocculation is well documented in terms of genetics and physiology, understanding of the biological mechanism of flocculation in complex and dynamic large scale brewing is still rudimentary. Additionally, as much research on yeast flocculation has been carried out at laboratory scale, extrapolation to industry scale is often challenging. Here, we give an overview of historical, as well as more recent, research on flocculation to elucidate the even more complex phenomenon of PYF.

The complexity of flocculation

Numerous flocculation theories and regulatory pathways have been proposed and reviewed (Verstrepen and Klis 2006; Soares 2011; Panteloglou et al. 2012; Stewart 2018). The currently accepted mechanism for yeast flocculation is the lectin-like theory, which involves lectin-like proteins (‘flocculins’), extending from the walls of flocculent cells. According to this model, flocculation is caused by the binding of flocculins to the carbohydrate residues of α-mannans, which act as receptors on surrounding cells (Miki et al. 1982). In this process, calcium ions are necessary to ensure the active conformation of the flocculins (Taylor and Orton 1975; Stratford 1989b; Goossens et al. 2015). Lectin-like flocculation is further sub-divided into two main phenotypes, Flo1 and NewFlo (Stratford and Assinder 1991). In the Flo1 group, flocculation is inhibited specifically by mannose sugars, whereas in the NewFlo group, flocculation is reversibly inhibited by various sugars, including mannose, glucose, sucrose and maltose (Ogata et al. 2021). Most brewing strains are of the NewFlo type (Sieiro et al. 1995), and do not flocculate before fermentable sugars have been exhausted, a feature that is of benefit to the brewing process.

The concentration of fermentable sugars plays a key role in initiating or inhibiting yeast flocculation (Soares and Duarte 2002), as the sugars are thought to compete with mannan residue receptors for flocculin binding sites (Stratford and Assinder 1991). Furthermore, transcriptional regulation of *FLO* genes is associated with the 'main glucose repression pathway' (Gancedo 1998). This pathway responds to the presence of fermentable sugar in the medium, where phosphorylation of glucose is initiated immediately after entering the cell. In the case of maltose or maltotriose, phosphorylation occurs after liberation of the monosaccharide by intracellular maltase. The phosphorylation process is followed by inactivation of the central SNF1 protein kinase, which represses the expression of *FLO* genes (Verstrepen and Klis 2006). Generally, the onset of flocculation in NewFlo strains of brewing yeast is triggered by sugar limitation (Smit et al. 1992; Sampermans et al. 2005; Speers et al. 2006), where the formation of flocculins is activated through *FLO* gene expression and, at the same time, the flocculin binding sites are exposed. However, the process may be more complicated in practice as several other signalling cascades are implicated in the control of yeast flocculation, such as Ras/cAMP and MAP kinase (MAPK) pathways (Madhani and Fink 1997; Rupp et al. 1999; Gagiano et al. 2002, 2003; Vyas et al. 2003; Schwartz and Madhani 2004; Kumawat and Tomar 2024). Further, Ogata (2012) demonstrated that the flocculation of lager yeast is under the control of a nitrogen catabolite repression (NCR)-like mechanism, as the expression of *Lg-FLO1* was found to be induced by starvation for nitrogen. Indeed, more recent research highlights the close connection between nitrogen starvation and yeast flocculation (Hou et al. 2023).

The complexity of flocculation lies not only in the intricate biochemical process by which flocculin-sugar interactions direct the formation of flocs, but also the impact of physical interactions (cell surface hydrophobicity, cell surface charge), which can act as non-specific determinants of flocculence (Jin and Speers 2000; Jin et al. 2001). The hydrophobic interaction theory of flocculation asserts that cell surface hydrophobicity is a major factor responsible for the flocculation of brewing yeast (Straver et al. 1993a, 1994, 1996), as the level of cell surface hydrophobicity was shown to have a strong correlation with the flocculence of brewing yeast (Akiyama-Jibiki et al. 1997; Jin et al. 2001). Likewise, the ability of brewing yeast strains to flocculate was reported to strongly correlate to the cell surface charge which is affected by the phosphorus surface concentration (Amory et al. 1988). The early hydrophobic interaction theory of flocculation failed to explain the specific requirement of calcium in floc formation or the influence of sugar. It did, however, imply a physical interaction being responsible for the flocculation process (Straver et al. 1993b). Likewise, flocculation properties were seen to be related to yeast strain and were genetically determined. Different yeast strains differ in terms of the conditions that induce flocculation and include cation content, temperature, pH and oxygen (Soares 2011).

Instability of flocculation in brewing yeast

Yeast flocculation is an unusually complex process but also an extremely unstable phenotype. Yeast cells experience numerous and diverse stresses during brewery fermentations (Gibson et al. 2007). This environment renders the yeast genome susceptible to mutation, which can result in changes in fermentation performance of brewing yeast strains. This is especially true for flocculation after multiple fermentation cycles (Stewart 2009). The *FLO* family consists of several *FLO* genes and these are unstable, causing great variation in flocculation behaviour between different yeast strains and across different generations of yeast (Reboredo et al. 1996; Sato et al. 2001). The specific architecture of *FLO* genes makes the flocculation potential of yeast strains susceptible to mutations.

The *FLO* genes were identified as one of the fastest evolving and expanding groups in the genome of *Saccharomyces cerevisiae* (Verstrepen et al. 2003). The *FLO* family includes *FLO1*, *FLO5* and *FLO9* genes that contain mega-satellites, highly repetitive DNA sequences of a 135 bp *FLO* motif (Richard and Dujon 2006; Rolland et al. 2010; Tekaia et al. 2013). The repetitive nature of internal tandem repeats within the *FLO* genes is a driving force for recombination events during DNA replication and this results in creation of various flocculation genes and corresponding flocculation properties (Verstrepen et al. 2004, 2005).

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The length of the *FLO1* mega-satellite has been positively correlated to the extent of flocculation (Verstrepen et al. 2005). However, a recent investigation demonstrated that replicative duplication or recombination slippage of a repetitive motif may be a rare event, as directed evolution experiments did not generate mega-satellite amplification (Saguez et al. 2022). The authors concluded that the chances of increasing mega-satellite mutations only happened under environmental conditions (high stress or lower temperature) where genomic replication was slowed down. It has also been demonstrated that the repeat motif inside the *FLO1* gene is not simply acting as a spacer of a given length, but rather the flocculation phenotype is dependent on the sequence of the mega-satellite (Saguez et al. 2022). While there is an increasing awareness that intragenic tandem repeat polymorphisms generate important functional variability, understanding of the biological role of intragenic tandem repeats is still underdeveloped.

It is important to note that the subtelomeric location of many *FLO* genes results in heterogeneous expression patterns. This heterogeneity is not only detected between strains, but also within a population of cells derived from a single clone (Halme et al. 2004). The various expression patterns (not always stochastic) result from epigenetic regulation of *FLO* genes. The epigenetic state of *FLO* genes is influenced by an alteration in chromatin structure near the telomeres, leading to the silencing of genes located in that region (Loney et al. 2009). This effect is heritable for many generations and can be long lasting (Stewart 2018).

Unstable and unpredictable flocculation is an important issue for industrial applications as a change in yeast flocculation will influence the fermentation process. This is especially the case when the brewing yeast undergoes serial re-pitching. A study carried out by Powell et al (2003), where age synchronised populations of a lager strain were prepared, showed that there was increased flocculation potential related to cell age. Sato et al (2001) reported that flocculation gradually decreased with successive generations and was associated with genetic change (partial deletion occurred in the middle region of the *Lg-FLO1*). However, the stability of yeast flocculation after serial re-pitching is proposed to be yeast strain dependent (Stewart 2018). Some strains did not show modifications of flocculation characteristics over the course of 98 or even 135 serial re-pitches (Powell and Diacetis 2012). These authors suggested that the lack of variation observed in some yeasts might be an artefact of the intrinsic flocculation characteristics, as yeast management and cropping regimes might have a greater influence in determining yeast flocculation during industrial brewing process.

Flocculation as a tightly regulated social behaviour

Yeast flocculation is a community building, cell aggregation trait that is one of the key mechanisms supporting yeast survival under unfavourable environmental conditions (Stratford 1992). The switch from a non-flocculent to flocculent state allows cells in the interior of the floc to be physically shielded from environmental stress by the outer cell layers. Upregulation of genes involved in stress resistance and multidrug transporters were observed with cells embedded within flocs (Smukalla et al. 2008). Yeast flocculation has been associated with the community level behaviour or ‘quorum sensing’ (Britton et al. 2021). This mechanism offers a significant advantage by allowing the population to adapt to environmental changes through a synchronised cooperative behaviour (Schuster et al. 2017; Padder et al. 2018). A known quorum sensing molecule, the aromatic alcohol tryptophol, was reported to induce strong flocculation in the diploid strain EM93 (Smukalla et al. 2008).

In industrial bioethanol fermentations with *S. cerevisiae*, yeast flocculation is induced by harsh conditions as a coping strategy (Westman and Franzén 2015). Brewing yeast strains flocculate under conditions of nutrient starvation, with nutrient limitation considered a form of stress during the later phase of brewing. Flocculation as a means of protecting cells from scarce nutrient availability, allowing them to survive has been demonstrated via experimental evolution in nutrient limited environments (Hope et al. 2017). The unstable nature of flocculation exemplifies the phenotypic plasticity of yeasts, allowing them to adapt quickly

to stressful environments and exploit new opportunities (Bouyx et al. 2021). Rossouw et al (2018) demonstrated that modifying the adhesive properties of *S. cerevisiae* through *FLO* expression could regulate the ecosystem dynamics of multispecies consortia. This implies that, when considering mixed fermentation scenarios, *FLO* genes play a pivotal role in the rapid adjustment to the challenges of interspecies competition. Recently, Pangestu et al (2024) demonstrated that the formation of flocs conferred protection against toxic chemicals (lactic acid and various lignocellulosic by-products) in the medium with the flocculating strain exhibiting an inherent ability to sustain high glycolytic activity.

Though knowledge of flocculation and its underlying mechanisms have been developed, it is difficult to control flocculation in practice. Indeed, flocculation is one of the most intriguing characteristics of industrial brewing for its complexity and strain dependent properties (Verstrepen et al. 2003). Considering the situation during industrial wort fermentation, there is typically amino nitrogen or fermentable sugar (maltose and maltotriose) left when yeast begin to flocculate. In the context of industrial brewing, flocculation may be affected by agitation or shear force caused by the evolution of CO_2 during fermentation (Speers et al. 2006). Sugar levels not only directly affect flocculation but also intensify fermentation shear force through metabolism and CO_2 production. On the other hand, flocculation induced by nitrogen starvation, as suggested by laboratory investigations, could represent a form of social protection during extreme stress conditions. However, with long term (serial batch), large scale fermentation, repitching (up to 100 generations) does not appear to have a significant effect on brewery fermentation (Powell and Diacetis 2012).

There are some indirect consequences seemingly related to the change in flocculation phenotype (fluctuation of yeast numbers in suspension) during long term, serial repitching in terms of flavour formation. For example changes in acetaldehyde concentration over > 100 production generations of serially re-pitched yeast (unpublished observations from the Tsingtao Brewing Co). This can be the result of the extremely high genetic instability of flocculation behaviour during the process of large scale production (Quain 2006). Only when the production yeast strain shows an unacceptable and irreversible change in beer flavour, is a new yeast batch propagated.

Premature yeast flocculation

Premature yeast flocculation (PYF) is an intermittent malt quality problem associated with susceptible yeast strains (Evans et al. 2023). Since the 1950s it has been reported by brewers that substances in wort can induce early flocculation of yeast (Axcell, 2002). However, the PYF inducing compounds have not yet been unambiguously identified, nor have the mechanisms been fully elucidated. Different investigations have led to different conclusions (Kudo 1958, 1959, 1960; Fujii and Horie 1975; Morimoto et al. 1975; Fujino and Yoshida 1976; Herrera and Axcell 1989, 1991a, b; Axcell et al. 2000; van Nierop et al. 2004, 2005). Accordingly, malt induced PYF is poorly defined, described as a rapid decline of yeast numbers in suspension during fermentation – but not necessarily early flocculation, but excessive sedimentation - despite the presence of sufficient nutrients (Lake and Speers 2008).

PYF negatively impacts on beer quality, with incomplete wort fermentation and undesirable changes in beer flavour (Axcell et al. 2000). It has been reported that there are different types of the PYF phenomenon. Premature flocculation during the primary fermentation (‘primary PYF’) results in under-attenuated beer with a decrease in real degree of fermentation (RDF) and accordingly lowered alcohol content. ‘Secondary PYF’ leads to sub-optimal yeast level during secondary fermentation (or maturation) resulting in a higher level of undesirable compounds, such as diacetyl (Evans and Kaur 2009; Vidgren and Londesborough 2011). Research by He et al (2022) reported another case of PYF which brought about a substantial shift in flavour profile through lowered secondary metabolite production, in particular of higher alcohols.

As brewing yeast is repitched to subsequent fermentations, exposure to PYF-positive (PYF^+) malt was demonstrated to have a long-term impact on yeast flocculation and fermentation performance (Panteloglou et al. 2012). Ultimately, PYF can cause production delays and financial losses to the brewer,

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as the beer may require additional blending or processing and, in severe cases, disposal (Koizumi et al. 2009; Kaur et al. 2012; MacIntosh et al. 2014).

Possible causes of PYF factor development

Biotic stress

PYF is a sporadic problem and is related to specific natches of malt. Although the mechanism of PYF is not fully understood, it is accepted that PYF is linked to microbial infection (bacteria, wild yeast and filamentous fungi) of barley and/or malt. The amount and the composition of the microbial population may vary with barley variety, growing region, and climate (Etchevers et al. 1977). A link has been suggested between PYF and harvest circumstances, particularly wet growing and harvest seasons, leading to an increased microbial load on barley (Kaur et al. 2012; Macintosh et al. 2014). Higher overall rainfall during the growing season, or during harvest, can dramatically increase the extent of microbial growth, especially of *Fusarium* species (van Nierop et al. 2006). However, two malt varieties monitored in Scotland showed no issue with PYF despite year round wet weather (Armstrong et al. 2018). Even though the climate of the growing region impacts on the microbial load on barley, it was suggested that the presence of microorganisms during malting may be more important than infection in the field (Lake and Speers 2008).

Indeed, significant enrichment of mycotoxin producing fung (*Fusarium* spp.) has been shown through the malting process (Chen et al. 2022). Increased microbial load during malting could influence the expression of PYF. Although bacteria are the predominant microorganisms on barley with certain bacteria shown to induce flocculation by bridging the lectins via the carbohydrates on the surface of the yeast cell wall (Zarattini et al. 1993), fungal infection is more likely to directly impact PYF. Filamentous fungi including *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, and *Rhizopus* spp. have been linked to the PYF phenomenon (van Nierop et al. 2004; MacIntosh et al. 2014). Direct inoculation has been used to test the initiation of PYF with van Nierop et al (2004) inoculating barley with *Aspergillus* species resulting in PYF levels comparable to that of the PYF$^+$ control.

MacIntosh et al (2014) demonstrated that PYF could be induced by infection with common barley fungal pathogens (*Fusarium graminearum* and *Cochliobolus sativus*) in the field between flowering and grain maturation. Further, through culture-independent, PCR fingerprinting techniques, the presence of yeast/yeast-like fungi was reported to relate to PYF$^+$ malts (Kaur et al. 2012). The authors also suggested that bacterial taxa were unlikely to be important in causing PYF (Kaur et al. 2012). Qin et al (2023) applied next-generation sequencing to explore fungal species in PYF barley malt. Even though they found high proportions of *Candida albicans* and *Gibberella baccata* in the PYF$^+$ malts, it is questionable whether the two species were linked to the occurrence of PYF. What is worth noting is the discovery of the decreased diversity of the fungal community within PYF$^+$ malts. More recently, Chen et al (2022), through the application of high-throughput sequencing, identified both fungal (*Aureobasidium* spp., *Candida* spp.) and bacterial (*Leuconostoc*) species that were correlated to PYF.

The discrepancies in putative PYF inducing microorganisms from different studies may be attributed to differences in detection methods and PYF samples. Research involving the deliberate inoculation of pathogens to barley or malt can determine a direct causative relationship between microbes and PYF. Studies on the detection of malt microbial communities exhibit an overview of changes in the diversity and composition of grain associated microbiota and provide potential insights regarding PYF.

Although there is consensus on the link between microbial infection of barley or malt and the PYF phenomenon, there is no universal acceptance of the microbial taxa which are responsible. It is plausible that undesirable microbial contamination which leads to a significant shift in composition of the microbial community can be associated with PYF. In other words, the presence of microorganisms may be symptomatic rather than causative. The complexity of the microbial communities on barley malt plays an active role in the malting ecosystem and malt properties (Laitila et al. 2007). Indeed, several yeasts showed strong antagonistic activity against field and storage moulds (Laitila 2007). Therefore, a diverse microbial community, consisting of a

variety of species, is important for barley malt to develop robustness and to deal with biotic stress confronted in the field or during the malting process.

Microbial colonisation of barley can induce plant-microbe interactions. On the one hand, the microbial populations secrete enzymes including endo-xylanase, β-glucanases and proteinases which break down the grain substrate for nutrient assimilation (Hoy et al. 1981; Kanauchi and Bamforth 2002; Schwarz et al. 2002). Many of these microorganisms also secrete antimicrobial factors, toxins, hormones and organic acids (Noots et al. 1999). Conversely, plants have their own innate immune system to defend against external challenges. When plant tissue is challenged, defence responses are activated (Saijo and Loo 2019). This includes the synthesis of chemicals such as alkaloids, unsaturated lactones, sulphur compounds, saponins, phenolic compounds, and the release of active oxygen and nitrogen species together with antimicrobial peptides and proteins to inhibit microbial growth (Saijo and Loo 2019; Ali and Baek 2020; Zhang et al. 2022). It has also been reported that barley produces a variety of defensins (small peptides) to inhibit the growth of a broad range of fungi (Thevissen et al. 2000). Currently, it has not been determined whether the barley defensive mechanisms or enzymatic breakdown by microbes contribute to the appearance of PYF. However, as will be discussed later, the production of the above compounds appears to correlate with the presence of PYF factors.

Abiotic stress

It has been reported that PYF status of malts is sensitive to operational conditions in maltings (Evans et al. 2023). Abiotic stress imposed on barley during malting, particularly steeping, has been shown to induce PYF, possibly reflecting the relatively high hydrostatic pressure on barley kernels. Indeed, high pressure treatment during steeping has been linked to PYF, with physiological restrictions on growth of barley rootlets, respiratory activity and secretion of gibberellic acid (Yoshida et al. 1979). Similarly, Axcell et al (1986) reported that a flocculation factor may be formed by forcing water into the grain at a late stage of steeping. They suggested that the osmotic pressure within the barley grain allowed leakage of the factor which was associated with barley husk.

The occurrence of PYF appears to be related to undesirable environments during barley growth or harvesting as well as during the malting process particularly steeping (Evans et al. 2023). Steeping conditions during malting provide an ideal environment for microorganisms to flourish on the barley grain (Axcell 2000; Laitila 2007) with both biotic and abiotic factors influencing the level of inocula inducing PYF. If such factors are generated before malting and already exist on barley, malting practices may exacerbate the potential for PYF. Further, barley may be disturbed simultaneously by biotic and abiotic stress, resulting in complex plant responses. Recent studies have shown that the response of plants to two or more stress conditions are interrelated in terms of the signalling pathways and cannot be extrapolated from the response of plants to stresses applied individually (Rodríguez-Vázquez and Carrieri 2024).

PYF-inducing compounds

PYF is caused by malt that contains compounds that are able to survive the brewing process and remain sufficiently active to trigger early flocculation of yeasts during fermentation (Lake and Speers 2008). The identity of these ‘PYF factors’ is not resolved and diverse compounds have been proposed. Indeed, the PYF factor may be a compound that occurs naturally in all malts, but is only active above a certain threshold. In this case, identification of a PYF-inducing compound is more complex than simply detecting it. This view is supported by the report that a putative PYF factor was detected on both both PYF negative (PYF^-) and PYF^+ malts, but at different concentrations (van Nierop et al. 2004).

Here, it is appropriate to provide an updated overview of research on PYF factors and various potential PYF factors segmented into three categories according to their chemical properties.

Polysaccharides with acidic nature

The working hypothesis for premature yeast flocculation is the lectin bridging mechanism where polysaccharides form cross bridges between yeast cells, forming larger than normal flocs and resulting in faster than normal sedimentation (Koizumi et al. 2008).

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Herrera and Axcell (1991a, b) reported the separation of a putative PYF factor - a high molecular weight (HMW) polysaccharide (> 100 kDa) - extracted from the malt husk. This was shown to be present at significantly higher concentrations in PYF⁺ wort and, as observed by electron microscopy, formed cross bridges between adjacent yeast cells. Sugar analysis of the HMW fraction showed the presence of arabinose, galactose, glucose, mannose, xylose, rhamnose and an acidic sugar component. A subsequent publication by van Nierop et al (2004) developed this and identified the sugars as arabinoxylan fragments from the husk. Moreover, they verified the inducibility of PYF factor through exposure of malt to extracellular fungal extracts or enzymatic degradation of the husk by commercial fungal xylanase prior to mashing. However, a commercial arabinoxylan (lacking glucuronic acid residues) only induced slight PYF at a high concentration (500 µg/mL) which supported the contribution of the acidic character of the PYF factor. Although they did not clarify the size of arabinoxylan fragment as an effective PYF factor, it can be speculated, based on medium sized arabinoxylan fragments being required, that excessive arabinoxylan degradation removed early flocculation (van Nierop et al. 2004). Koizumi et al (2009) also confirmed the PYF factor as polysaccharide in nature, but with lower molecular weight (<40 kDa) and composed of arabinose, xylose, galactose, rhamnose and galacturonic acid. In this case, severe PYF was induced by as little as 0.3 µg/mL of concentrated polysaccharides. Interestingly, the HMW polysaccharide is not required for PYF, as digestion by commercial enzymes into fragments of less than 5 kDa did not prevent PYF activity (Koizumi et al. 2008). Further work indicated that glucuronic acid residues were the specific recognition factor facilitating interaction with developing yeast flocs (Koizumi et al. 2009).

The mechanism of generation and the characteristics of polysaccharides as PYF factors were reported in an investigation into the secretome (secreted proteins) of *Fusarium graminearum* inoculated to the barley husk (Xie et al. 2022). Many of the extracellular enzymes produced by *F. graminearum* are directly involved in the degradation of the barley husk. Of the proteins in the secretome, hemicellulases were the most abundant. These included a combination of main and side chain degrading enzymes breaking down husk hemicellulose. Polysaccharides, with a range of molecular weights, obtained from barley husk inoculated with *F. graminearum* were assessed for their impact on PYF (Xie et al. 2022). Using ultrafiltration, fractions of acidic polysaccharides with a high molecular weight (> 10 kDa) were shown to play a role in inducing PYF at low concentration.

Although the implications of polysaccharides with a range of molecular weights in brewing are largely unknown, it is generally accepted that the extracellular enzymes secreted by fungi which degrade the husk into acidic polysaccharides are a likely cause of PYF.

Antimicrobial peptides

An alternative hypothesis is that PYF is a result of antimicrobial peptides produced during microbial colonisation that negatively affect yeast metabolism. Plants have evolved a variety of mechanisms to defend against insects and microorganisms, including the synthesis of antimicrobial factors, typically peptides (Gorjanović et al. 2004). Further, microorganisms colonising barley can produce antimicrobial factors to enhance their competitive advantage (van Nierop et al. 2006). Generally, antimicrobial factors from plants are active against a broader range of organisms compared to those produced by bacteria or fungi. As PYF malts are usually associated with the microbial infection of barley, production of antimicrobial factors, particularly anti-yeast compounds, may pose a threat to yeast metabolism during fermentation. This would lead to a range of brewing defects, including PYF (van Nierop et al. 2008). Despite the presence of antimicrobial factors in barley to defend against microbial attack or injury (Leah et al. 1991; Broekaert et al. 1992, 1995; Molina and García-Olmedo 1993; Molina et al. 1993), their possible role(s) in the brewing process have not been fully elucidated (van Nierop et al. 2006).

The antimicrobial peptides isolated from plants contain cysteine residues and can form stable disulphide bonds (van Nierop et al. 2006) with thionins and non-specific lipid transfer protein (ns-LTP) being the most widely reported (Florack et al. 1994). The interaction of antimicrobial peptides with the plasma membrane of microorganisms is

implicated in their toxic effect (Broekaert et al. 1997). Early research by Okado et al (1970) reported laboratory evidence of the lethal effect of a toxic substance from wheat and barley on brewing yeasts at a concentration of 4 µg/L with suppression of sugar uptake at low concentrations (ten-fold dilution) without cell death. Axcell et al (2000) suggested that this toxic compound could be the ns-LTP described by Okado et al (1970) and further hypothesised that this might cause the yeast to prematurely flocculate via charge interaction. Subsequently, Gorjanović et al (2004) reported that ns-LTP from malting barley *in vitro* inhibited fermentation and respiration of *S. cerevisiae* via leakage of cell constituents. However, the inhibitory effect of ns-LTP on yeast fermentation was not observed in the brewing process (Gorjanović et al. 2004). Similarly, Cvetković et al (1997) noted that the activity of the pathogenesis-related proteins isolated from barley was lost during the brewing process due to thermal denaturation (Cvetković et al. 1997). Contrary to Gorjanović et al (2004), van Nierop (2005) noted the identification of ns-LTP as the antimicrobial activity in malt which was found to survive the brewing process. Additionally, van Nierop et al (2008) reported a 96-well microplate antimicrobial assay for the determination of anti-yeast activity from extracts of malt. Interestingly, elevated antimicrobial peptides were found in PYF$^+$ samples, but also in malts exhibiting gushing activity (van Nierop et al. 2008).

Antimicrobial peptides have not been detected in PYF$^+$ malt in Canada or the United Kingdom and appear to be a rare occurrence (Lake and Speers 2008; Porter et al. 2010; Armstrong et al. 2018). In addition, it has been reported that the putative presence of membrane disruptive antimicrobial peptides in PYF$^+$ samples was inconsistent with the observation that sugar uptake by yeast was not significantly affected by PYF (Panteloglou et al. 2013). These observations indicate that antimicrobial peptides are inconsistent with being the ‘PYF factor’, but maybe another symptom of microbial infection.

Phenolic compounds

Phenolic compounds have been implicated in PYF. They represent a significant part of the secondary metabolites of plants with many involved in defence mechanisms (Badea et al. 2023). Although two forms of phenolic compounds (free and bound) are found in cereals, the majority are in the bound form, conjugated to polysaccharides and incorporated in cell walls (Adom and Liu 2002; Nardini and Ghiselli 2004; Das and Singh 2015; Chtioui et al. 2022). Of these, ferulic acid is abundant in cereals and is reported to inhibit growth of fungi such as *F. graminearum* (Ponts et al. 2011). The presence of ferulic acid in barley arabinoxylans is well known. This phenolic acid is covalently linked to arabinose residues in the xylan backbone via an ester bond (Kroon et al. 1999). However, substantial differences in the frequency of ferulic acid substitutions in arabinoxylan backbones were observed, which may arguably result from differences in detection methods (Dervilly-Pinel et al. 2001; Adams et al. 2005; Sørensen et al. 2007). Ferulic acid can be liberated from the bound form via feruloyl esterase, which hydrolyses the ester bonds that link ferulic acid to arabinoxylan. The amount of free ferulic acid in standard Congress worts ranges from 1.9-2.8 mg/L (Coghe et al. 2004), but increases to 13.9 mg/L with hydrolysis (Nardini and Ghiselli 2004).

Until the report from Fujino and Yoshida (1976), ferulic acid had received little attention regarding PYF. They argued for a direct role of arabinoxylan in PYF, with ferulic acid associated with the active component of the PYF fraction from malt. This idea was developed by Lake (2008) who reported the minimal effect of free ferulic acid on PYF. Although the concentration of ferulic acid (75 mg/L) was considerably higher than the free levels in normal wort (1.9-2.8 mg/L), Lake (2008) suggested that ferulic acid bound arabinoxylan exhibited some PYF-like activity, as the activity of the 100kDa retentate from a PYF sample was reduced via mild hydrolysis prior to inoculation into control wort. Benabdesselam (2010) working with *Saccharomyces uvarum*, noted that flocculation was not observed with Sympa or Nymphea malt, but was observed with Trumpf malt. Analysis of an ethanol precipitate of the Trumpf malt suggested that the fraction of malt impacting on the flocculation of *S. uvarum* was a consequence of ester bonding between sugars and phenolic acids. This was emphasised by the loss of flocculation when the precipitate of the Trumpf malt was treated with trypsin or esterase but not glucanase, proteinase, cellulase or amylase. The

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presence of phenolic acids (ferulic acid, isoferulic, paracoumaric, vanilic and syringic acids) was also confirmed by thin layer chromatography (Benabdesselam 2010). Shang et al (2015) compared the concentration of both arabinoxylan and bound ferulic acid from PYF$^+$ and PYF$^-$ worts, made from the same barley cultivar by one maltster. They suggested that a specific molecular weight fraction of arabinoxylan and bound ferulic acid were essential for PYF induction.

It seems clear that phenolic acids or at least bound ferulic acid are implicated in the induction of PYF. However, the mechanism of how a complex of ferulic acid bound arabinoxylan would initiate PYF is still not clear. It is of note, that water soluble arabinoxylan forms highly viscous solutions, forming gels by covalent cross-linking of arabinoxylan chains via dimerisation of ferulic acid in the presence of hydrogen peroxide/peroxidase (Izydorczyk et al. 1990). Further, the gelling potential of arabinoxylan depends on the amounts of ferulic acid substitution and distribution along the backbone chain (Dervilly-Pinel et al. 2001). Arabinoxylan fractions with high ferulic acid content, high molecular weight, and relatively unsubstituted xylan backbone are capable of extensive cross-linking and formation of well developed networks (Izydorczyk et al. 1992). From the view of a barley and malt defence system, it is well known that plant tissue at high risk of infection may constitutively contain phenolic compounds and active forms of oxygen (and hydrogen peroxide), which inhibit microbial growth (Singh et al. 1972; García-Olmedo et al. 2001). Consequently, the accumulation of both hydrogen peroxide and ferulic acid might improve the potential for cross linking and polysaccharide-yeast interaction.

With regard to yeast, some brewing strains of *S. cerevisiae* are able to convert ferulic acid to 4-vinylguaiacol via the enzyme ferulate decarboxylase (Goodey and Tubb 1982). 4-vinylguaiacol is less toxic than ferulic acid and confers a characteristic aroma to beer referred to as a 'phenolic off-flavour' or POF. It would be of interest to determine whether POF positive and negative yeast strains differ in their sensitivity to PYF malt.

Debate on the malt-induced PYF factors and related theories is ongoing and there is yet no consensus. This may be related to different PYF malt samples and experimental conditions being applied by different research groups. The possible existence of diverse root causes for the PYF phenomenon and several factors related to different types of PYF malts, which may act individually or in concert, illustrates the complexity of premature yeast flocculation.

Methods for detection of PYF

It is recognised that mash conversion and lauter time of (what becomes) PYF$^+$ malt are normal, and that there are no PYF warning signs in the brewhouse before fermentation. Furthermore, malt specifications are typically normal and consistent with standard targets (Armstrong and Bendiak 2007). Besdides, it is not just the problem of the presence (PYF$^+$) or absence (PYF$^-$) of PYF factor but the existence of malts with varying PYF potential makes detection more complicated. Therefore, there is a need for reliable tests for the early detection of PYF$^+$ malts.

MacIntosh and colleagues assessed barley malts with fungal infection in the field (MacIntosh et al. 2014), and the presence of excess free amino acids (FAN) was observed in PYF worts. However, PYF$^+$ worts from the work of Panteloglou et al (2013) showed similar levels of fermentable sugars and FAN compared to PYF$^-$ worts. Such inconsistencies in the concentration of FAN may result from different types of PYF malt used in the respective studies and the possibility of different PYF factors which may complicate the detection of PYF. In addition, as with potential PYF malts from breweries, there are strict specifications for malt quality. If the FAN value is above the specification, process adjustments can be made accordingly. Therefore, it is difficult to use FAN content to identify malts that possibly confer PYF.

After more than half a century of research, the lack of a standard protocol hinders the definitive quantification of PYF malt. Summaries of the various diagnostic approaches to quantify PYF are given in Table 1. As there is no universal acknowledgement of the nature of the PYF factors, fermentation performance based indicators (such as suspended yeast) are commonly employed to determine the PYF potential of malts by maltsters and brewers. The EBC 2L scale tall tube fermentations suggested

Table 1.

Detection methods for PYF

Methods		Assay size	Duration	Limitations	Reference
Small-scale fermentation test	Prediction of malt quality based on malt fermentability	2 L fermentation 'tall' tubes	8 days	Time consuming and labour intensive, in addition to requiring greater amounts of raw material especially for those fermented in larger size	Kruger et al. 1982
	13°P all-malt wort fermented with standardised yeast (grown on defined media)	100 mL separation funnels	70~80 h		van Nierop et al. 2004
	EBC Congress wort supplemented with 4% glucose at a fermentation temperature of 21°C in a water bath	50 mL measuring cylinders	48 h		Jibiki et al. 2006
	11.1°P wort added with 4% glucose and fermented with SMA yeast at 1.5 × 10^7 cells/mL at a minimum volume of 15 mL	15 mL test tubes	48 h		Lake et al. 2008
	Supplementing wort with 6 mg/L linoleic acid and using a highly flocculent PYF sensitive yeast strain for fermentability test	250 mL dropping funnels	40 h		Panteloglou et al. 2010
	Yeast-14	15 mL tubes	78 h		ASBC, 2011
Extraction of 'PYF factors'	Extraction of PYF factors from barley or malt samples + Test the PYF activity with yeast cells	4 mL cuvettes	3 h	/	Koizumi and Ogawa 2005
	Extraction of the anti-yeast compounds from barley malt + Quantification of the anti-yeast activity with yeast cells	96 wells microtitre plate	/	As malts associated with PYF and gushing activity had high anti-yeast activity, it was therefore proposed that the assay may be beneficial as a malt analysis to predict possible fermentation issues rather than PYF detection	van Nierop et al. 2008
Microbial populations screening	Direct monitoring of barley malt samples using molecular methods (T-RFLP) to distinguish PYF$^+$ samples in accordance with the DNA fingerprint patterns of 360-460 bp *HaeIII* TRFs	centrifuge tubes	/	The identification of TRFs was tentative and might not be complete as only three clone libraries were constructed	Kaur et al. 2012
Prediction model	Yeast-in-suspension data were modelled by a 'tilted' Gaussian fit, and attenuation curves were prepared using a logistic model	See Yeast-14	See Yeast-14	Extra sampling needed when population peaked to increase the model accuracy, which requires reevaluation on a case-by-case basis; also, fermentation based	Armstrong et al. 2018
	Proposed five simpler models to describe fermentation dynamics and identify PYF fermentations in conjunction with the ASBC Yeast-14 method, with 2-parameter gamma model giving the most reliable estimate in a brewery setting	See Yeast-14	See Yeast-14		Rudolph et al. 2020

by Kruger et al (1982) are time consuming, requiring significant amounts of malt, and are difficult to apply to multiple trials. Development of miniature fermentation systems which downscale the process have been hampered by issues with reproducibility and inconsistency. To address this, the American Society of Brewing Chemists (2011) established a standard method Yeast-14 to distinguish PYF$^+$ malts from normal malts through standardised mashing regimes, small scale fermentation (15mL volume) and use of a specific yeast strain.

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Alternative detection methods, which are independent of fermentation, have also been proposed. Despite the lack of consensus on PYF factors, they are commonly water washable and amphipathic. Attempts have been reported (Koizumi and Ogawa 2005) to extract PYF factors from malts, followed by monitoring the impact of factors on a suspended yeast concentration against a control after a three-minute period. Anti-yeast activity of malt was also described, and the assay was suggested to provide an indicative measurement of malt quality, including that of PYF potential (van Nierop et al. 2008). Further, a molecular method (terminal restriction fragment length polymorphism or TRFLP) was suggested as a potentially effective route to detect PYF based on the screening of microbial populations of barley malt (Kaur et al. 2012). Differences were reported for terminal restriction fragments of fungi associated with PYF$^+$ and PYF$^-$ malt. However, the main focus of this work is the identification of potential PYF associated microbes within the PYF samples. Whilst promising insight, care should be taken when extrapolating results of terminal restriction fragments to other barley malt samples.

Since PYF alters the kinetics of yeast cells in suspension, the feasibility of using mathematical modelling to describe yeast dynamics in abnormal fermentations was evaluated to improve the detection of PYF (Armstrong et al. 2018; Rudolph et al. 2020). A pioneering investigation by Armstrong et al (2018) developed a predictive model to determine whether the malt could cause PYF, work which triggered an extension of the ASBC miniature fermentation assay (Yeast-14) test for the presence of PYF$^+$ malt. Subsequently, Rudolph et al (2020) provided guidelines to detect PYF using the Gamma model in conjunction with the ASBC Yeast-14 method, or by using linear discriminant analysis (LDA) to detect aberrant fermentations in industrial settings.

It is noteworthy that there are differences in the susceptibility of yeast strains to PYF (Kruger et al. 1982), and that they behave differently when exposed to wort from PYF$^+$ malts. Generally, ale yeast strains, either flocculent or non-flocculent, were less sensitive to PYF$^+$ malts; while lager yeast was sensitive to PYF$^+$ malt, even when non-flocculent in nature (Jibiki et al. 2006). Further, crops of the same lager yeast from different breweries showed clear differences in their sensitivity to PYF$^+$ malts (Jibiki et al. 2006). Therefore, it is important for maltsters, to have a PYF-sensitive yeast strain to identify the PYF issue. Breweries may use their production yeast for comparative analysis of malt quality.

So far, fermentation tests are considered the most definitive and widely used method for identifying PYF malts (Kaur et al. 2012), even if they cannot determine the compounds causing PYF, and risk misidentifying samples without PYF factors as PYF$^+$ (Lake and Speers 2008). The results obtained from the fermentability test can nevertheless give useful information about potential problematic malts.

Strategies to avoid or alleviate PYF

Premature yeast flocculation originates from poor quality malts, particularly those associated with microbial contamination in the field. Multiple strategies are available for controlling malting barley associated pathogens through cultural practices, chemical and biological controls (Cheremisinov and Rengarten 2024; Gabriel et al. 2024; Krnjaja et al. 2024). As microbial contamination is weather dependent, it is anticipated - with current climate change scenarios - to become more complicated to initiate adaptation strategies in the field. Therefore, the scope of the following discussion is restricted to strategies that could be implemented by maltsters or brewers.

Various practical applications to mitigate PYF issues have been proposed during the past decades. Although increasing wort oxygen and trace nutrients in wort have proven beneficial in managing high gravity fermentation, they were not successful in overcoming PYF (Armstrong and Bendiak 2007). In general, different strategies are proposed from three perspectives to handle the problem; these are summarised in Table 2.

PYF factors can be recovered by water and they - and the microbial load on the grain - can be removed from the surface by washing or rinsing (Axcell et al. 2000; Jibiki et al. 2006). In the more recent publication, distilled water was added to PYF$^+$ malt and shaken for four hours before wort preparation (Jibiki et al. 2006). Even though washing decreased

the PYF potential, the improvement in suspended yeast cell number at the end of fermentation was only half that of the PYF⁻ control.

Maintaining aerobic conditions (with low CO_2) during malting may play a role in lowering PYF potential. Other practical strategies available to brewers include blending PYF⁺ malt with PYF⁻ malt to reduce the problem to a manageable level. However, it is worth noting that when the PYF problem was severe, blending was unacceptable, even if only a small amount of PYF⁺ malt was used. Shang et al (2022) applied a recombinant xylanase inhibitor to mitigate the effects of PYF factors during mashing but with little inhibitory effect.

Several other ways have been proposed to solve the issue from the perspective of the yeast. Notably, rousing the yeast after initiation of PYF – which is a challenge in terms of CO_2 breakout - did not alleviate the problem (Armstrong and Bendiak 2007). However, increasing the yeast pitching rate was sometimes successful in minimising the observable effects (Axcell et al. 2000). In this context, an effective strategy may be dependent both on the yeast strain and the type of PYF encountered.

Beyond all the strategies discussed above and listed in Table 2, Armstrong and Bendiak (2007) suggested that new malting barley varieties may be a solution for preventing PYF. Such an approach was also considered by Armstrong et al (2018) in their work with Scottish malts. Even though, year-round wet weather is observed in Scotland, the malt varieties used there have adapted to the wet climate and developed resistance to fungal infection.

It can be concluded that any strategies that result in reducing microbial load on the grain or abiotic pressures during the malting process would be effective practices to be used by the brewing industry for prevention of PYF factor development. What is more, malt properties could be improved by controlling the microbial population during malting in a way that encourages the growth of beneficial microorganisms and, at the same time, inhibits harmful microbes such as *Fusarium* species (Laitila 2007). In this sense, biocontrol could also be a practical alternative for lowering the risks of PYF.

PYF in the brewing industry

The currently evolving climate situation significantly threatens global food supply and food security by reducing both crop yield and quality, with barley being no exception. The frequency of extreme weather events (documented worldwide with more than 9135 events during the last three decades) has increased globally due to climate change (Wang et al. 2022).

It has been predicted that the annual global barley yield will decline by up to 17% due to more frequent and extreme weather events (Xie et al. 2018), causing ongoing concern about the sustainability of world barley production. The larger decreases in barley supply are predicted to occur in China, Japan and Belgium (Xie et al. 2018). In addition, the intricate interplay between climate, plant, and microflora not only causes economic issues such as PYF but generally results in enormous impact on food security with, for example, mycotoxin associated safety issues. Driven by many factors, including the climate crisis, the brewing industry is also facing geopolitical tensions and challenges. Regulations of the trade in barley import and export, such as tariff policies, are often shaped by geopolitical forces, and vary significantly between countries and regions. This ever-changing geopolitical dynamic has significant sway on the brewing industry, resulting in a less robust and resilient environment for the availability and quality of brewing raw materials.

Many brewers around the world have problems with PYF. Even if considered as an intermittent occurrence, it is an industrially significant issue for both maltsters and brewers. Since the late 1950s, the Kirin Brewery Company has pioneered numerous in-depth studies on PYF (Fujii and Horie 1975; Fujino and Yoshida 1976). Subsequently, another Japanese company reported research on the topic (Okada et al. 1970). In the late 1980s, South African Breweries (SAB) also experienced the issue and published much research about PYF (Axcell et al. 2000). Other investigations from Canadian breweries were also shared in publications (Armstrong and Bendiak 2007; Rudolph et al. 2020).

Both barley growing conditions and the malting process are important for production of high quality

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Table 2.

Potential strategies to alleviate PYF

Strategies		Limitations	Reference
Reduce the microbial load entering the brewing process	Surface washing and drying of malts prior to mashing	Only partially improves the suspended yeast cell counts (around 50% of the PYF⁻ control)	Jibiki et al. 2006
	Removal of malt husks prior to mashing	Removal of malt husks prior to mashing has an impact on wort filtration process and wort quality. Besides, this practice had little effect on certain PYF⁺ malts (unpublished personal observation from the Tsingtao Brewing Co.)	Van Nierop et al. 2004
	Wet milling of malt and discarding the steeping water before mashing	Reduce wort extract	Axcell et al. 2000
	Surface washing prior to steeping	Only able to reduce the microbial load on the grain surface, little effect on internal microbial and those infected during malting	Panteloglou et al. 2012
	Spraying the grain samples with neutral electrolyzed oxidising water	At the risk of residue presence which can affect fermentation; Increase the cost to breweries	Guo et al. 2020
	Adding lactic acid bacteria to steeping water	Reported with restricted growth of *Fusarium*. Not tested with PYF potential	Laitila 2007
Dilution of PYF factors/effects during the brewing process	Blending PYF⁺ malts with PYF⁻ during mashing	Limited ratios of PYF⁺ malt could be utilised, highly dependent upon the specific PYF potential of the PYF+ malt	Jibiki et al. 2006
	Addition of xylanase inhibitor during mashing	Only inhibits the production of PYF factor during mashing, but not the PYF factor originated from barley and malting, thus the inhibitory effect on PYF⁺ malt is not significant	Shang et al. 2022
Improvement from the yeast side	Addition of tannic acid (which is hypothetically bound to the yeast cell's surface to suppress flocculation) during the fermentation process	At Tsingtao Brewing Co. it has not been possible to reproduce the effect on lowering residual extract with additional tannic acid, which only improves the level of cell-in-suspension and final concentration of acetaldehyde, but shows no improvement on final gravity (unpublished personal observation from the Tsingtao Brewing Co.)	Guo et al. 2020
	Increasing yeast pitching rate	Depends on the type of PYF encountered and doesn't apply to all instances	Axcell et al. 2000

malts. Although PYF issues are seen in the brewery, for those larger brewiong companies who have their own malting facilities, more consideration should be given to the source of barley and the malting process. Malting barley crop year conditions play a significant role in malting quality and brewing. Also, each malthouse produces malt with slightly different characteristics from similar barley harvests (Armstrong and Bendiak, 2007). As a result, PYF is generally related to a particular crop year and can occur in malt from particular malthouses. As it is commonly accepted that PYF is linked to microbial infection of barley and/or malt, the management of the microbial load on the barley is a primary task for the malthouse. In particular, special attention should be ginen to the occurrence of *Fusarium*, as its presence has been attributed to many brewing problems, including PYF (van Nierop et al. 2004; Sarlin et al. 2012; Jin et al. 2021).

In addition to the growth of microorganisms on the grain in the field, growth occurs during malting. As

suggested by Chen et al (2022), the microbes from the barley grains undergo proliferation at different rates during the malting process. A recent study indicated that *Fusarium* spp., originally located on the husk of barley grains, can continue to grow their hyphae into the endosperm and embryo during the malting process (Jin et al. 2021). This also implies that once *Fusarium* infection occurs, it is difficult to wash out the pathogen. In this case, the malting process is of great significance as it may either exacerbate or alleviate the problem.

If the malting conditions suppress the growth of problematic microorganisms, the malthouse may protect itself from development of PYF^{+} malt. On the other hand, if the environment of the malthouse encourages the growth of specific microorganisms, the potential for PYF increases. In practice, some malting plants produce malts that exhibit PYF, whereas others do not, despite using the same barley (Armstrong and Bendiak 2007). Further, the same report, noted that during the malting process, steeping appears to be the process in which most of the problems develop.

Challenges and prospects

The incomplete understanding of premature yeast flocculation originates from the fundamental question, what exactly is PYF and what are the inducing compounds? This limited understanding also hampers the development of effective detection and control over PYF. A positive development is that research on PYF has now entered the 'omics' stage (He et al. 2021; Chen et al. 2022; Xie et al. 2022; Qin et al. 2023). This will deepen insight into the fundamental nature of PYF and, potentially, will allow effective management during the malting process. Although several PYF related microorganisms have been suggested, it is still challenging to conclusively associate specific microbes with PYF. Future work on integrating metagenomics and metabolomics will assist in identification of potential PYF factors, and clarification of how these are generated through microbial infection. As poor quality malts with microbial infection may also adversely impact the fermentation process, they are not necessarily associated with the PYF issue. Therefore, more carefully designed experimental work is needed to probe the causes of PYF and differentiate these causes from other features of poor quality malts. To date, most studies on PYF have focused on the external changes associated with microbial infection. Research to address the internal mechanisms regarding plant immune or defensive systems may lead to a greater overall understanding of PYF.

PYF events and related research are occasionally reported in the literature. However, it is likely that the extent and frequency of occurrence in the brewing industry is far more extensive than suggested from academic reports. The challenges ahead can be eased through integration of advanced knowledge at industry levels and communication between maltsters, brewers and researchers for the combined benefit of the industry. Over time, better understanding of the PYF issues will facilitate more robust strategies to cope with effects of climate change and ensure more consistent malt quality, even in rapidly changing environments.

Author contributions

Brian Gibson and **Yang He** contributed equally to conceptualisation, writing, reviewing and editing the manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest.

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