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Malt induced premature yeast flocculation: its origins, detection and impacts upon fermentation

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1. Abstract

Premature yeast flocculation (PYF) is a sporadic problem encountered during industrial brewing fermentations. Current hypothesis states that factors thought to arise from fungal infection of the barley in the field and/or the malt in the maltings cause yeast to flocculate prematurely and/or heavily before the depletion of the sugars in the wort. This results in poorly attenuated worts, with higher residual extract and lower alcohol by volume, flavour abnormalities (i.e. diacetyl, SO_2), lower carbonation levels, disruption of process cycle times and potential issues with the re-use of the yeast in subsequent fermentations. Consequently, premature yeast flocculation generates significant financial and logistical problems both to the brewer and the maltster.

In the current study, a small-scale fermentation assay was developed and optimised to predict the PYF potential of malts, as well as to investigate the importance of the yeast strain in the incidence and severity of the phenomenon. Furthermore, the impacts of the PYF factor(s) (i.e. arabinoxylans, antimicrobial peptides) on yeast fermentation performance and metabolite uptake were also studied, whilst the Biolog detection system was investigated as a potential rapid tool to detect PYF.

The results obtained suggested that our in-house assay can be successfully used to predict the PYF potential of malts 69 or 40 h post-pitching depending upon the yeast strain used. Whilst ale yeasts were not found to be susceptible to PYF, lager yeasts exhibited different degrees of susceptibility, even to the same PYF factor(s). More specifically, the more flocculent lager yeast SMA was found to be more susceptible than the medium flocculent lager yeast W34/70. However, interestingly, the fermentation performance of a PYF+ wort could be significantly improved by using a non-flocculent and a relatively PYF-insensitive lager yeast. It was also shown that worts with lower amount of glucose and maltose could be responsible for poor fermentation profiles and/or heavy PYF as well as elevated residual sugars and lower fermentability. The observation that linoleic acid (6 mg.l-1) exacerbated PYF ($P = 0.047$) and made its detection more rapid was found to be contrary to the “titration hypothesis” (Axcell *et al*., 2000) which hypothesised that the addition of fatty acids might “titrate” out antimicrobial peptides so that they can no longer bind to the yeast cells. High gravity fermentations with worts inducing PYF did not have a significant effect ($P > 0.05$) on yeast physiological characteristics or fermentation performance, suggesting that the PYF+ sample used in this study was inducing PYF though the ‘bridging’ polysaccharide mechanism rather than through the antimicrobial peptides. The Biolog system can be used for the

metabolic characterisation of different flocculence lager yeasts incubated in different fermentation media, whilst wort composition had a significant effect in redox reduction reactions.

2. Conclusion

The objective of this thesis was the study of the PYF phenomenon in the brewing and malting industry. The aim was to investigate the origins, detection and impacts of the PYF factor(s) upon fermentation. To achieve this, several steps were undertaken. These steps included the development and optimisation of a small-scale fermentation assay to predict the PYF potential of malts, the study of how yeast strains of varying degrees of flocculence are impacted by PYF, the investigation of the impacts of PYF factor(s) on fermentation performance and metabolite profile as well as the study of sensitivity of different yeast strains against PYF factor(s) (i.e. PYF+ worts).

Using the in-house small-scale fermentation assay, the medium (W34/70) and highly flocculent (SMA) lager yeast strains, the PYF potential of the malts was successfully predicted 69 and 40 h post-pitching, respectively. (Panteloglou *et al.*, 2010). SMA yeast was found to be more susceptible to PYF factor(s) than W34/70 yeast, (Panteloglou *et al.,* 2011), supporting the previous findings of Armstrong and Bendiak (2007) who indicated that the more flocculent lager yeast strains were more susceptible to PYF, whilst a range of PYF+ malts sourced from the industry exhibited different degrees of PYF severity when fermented with the same brewing lager yeast strain. This result was found to be in agreement with earlier studies suggesting that there are varying types of PYF factor(s) and consequently different degrees of PYF (Van Nierop *et al.*, 2004). The fact that the results obtained from our in-house PYF assay were in agreement with the results obtained from the majority (80%) of the research labs who participated in a ring-trial in a collaborative study between research labs worldwide convened by Campden BRI, indicated that our in-house small-scale fermentation assay (Panteloglou *et al.*, 2010) can be successfully used for the prediction of the PYF potential of different malt samples. The results obtained were consistent with the PYF problems that had been presented by the malts when brewed on an industrial scale. Besides that, it was also concluded that worts containing lower amount of glucose and maltose could be responsible for poor fermentation profiles, heavy and/or PYF, as well as elevated residual sugars and lower fermentability at the end of the primary fermentation. These findings supported the view of Axcell (2003) who highlighted the importance of wort composition both on yeast flocculation and fermentation performance.

In order to achieve a reduction in the time required for detection, as well as to enhance the current knowledge of the mechanisms involved in the PYF process, our in-house fermentation

assay was optimised. The results obtained suggested that supplementation of the worts with 6 $mg.l^{-1}$ linoleic acid ($C_{18}H_{32}O_2$; 18:2) before pitching, as well as the use of the highly flocculent PYF sensitive lager yeast strain SMA, enabled the differentiation between PYF+ and PYF- malts just after 40 h post-pitching. This result was found to be in agreement with the findings of Jibiki *et al.* (2006) who, by using a different fermentation PYF test (i.e. 50 ml test tube), lower pitching rate (i.e. 15×10^6 live $cells.ml^{-1}$ instead of 20 million cells) but the same yeast strain (SMA), also showed maximum differences in the number of suspended yeast cell counts between PYF+ and PYF- fermentations at the same time point through fermentation (i.e. 40 h post-pitching). The results obtained also indicated that among the five experimental factors used to optimise the PYF test (i.e. $CaCl_2$, Zn^{2+}, 18:2, glucose and "turbid" worts), chosen on the basis that they would affect flocculation, only the addition of 18:2 had a significant effect. This effect was possibly because solid particles (i.e. 18:2) act as nucleation sites for CO_2 bubble formation allowing the increase of suspended cells, due to lower CO_2 accumulation in the fermenting broth, and therefore promoting a more vigorous fermentation (Boswell *et al.*, 2002; Stewart & Martin, 2004; Kuhbech *et al.*, 2007; Gibson, 2011). However, since the production of "turbid worts" had no impact on the ability of the test to distinguish between PYF+ and PYF- worts, if the nucleation hypothesis is correct, then it is something very limited to the lipid content of the nucleation sites.

Using the in-house small-scale fermentation tests, the importance of varying degrees of flocculence of lager and ale yeast strains on the incidence and severity of the PYF phenomenon was also investigated. The results obtained suggested that the yeast strain has an important role on the PYF phenomenon. Thus, whilst none of the ale yeasts (i.e. NCYC 1332, M2) used in this study were found to be susceptible to the different PYF factor(s), lager yeasts (i.e. W34/70, SMA and 'Industrial') exhibited different degrees of susceptibility even to the same PYF factor(s). More specifically, it was found that the more flocculent yeast, SMA, exhibited a higher degree of susceptibility than the less-flocculent yeast, W34/70. This result was found to be in agreement with previous studies indicating that ale yeasts, either flocculent or non-flocculent, were not susceptible to PYF (Jibiki *et al.*, 2006). Interestingly, it was also shown that the fermentation performance of a PYF+ wort could be significantly improved, with respect to the number of suspended yeast cell counts, residual gravity and alcohol yield, by using a non-flocculent lager yeast strain which is relatively insensitive to PYF (Panteloglou *et al.*, 2011). However, the improvement in the fermentation profiles varied amongst the different PYF+ samples. These results help to explain why malt supplied from the same producer (i.e. barley from the same variety, harvest year and region of production) and malted under the same conditions can give rise either to 'normal' or PYF worts. Thus, besides the PYF potential of the barley/malt samples, the yeast strain was found to have an important role on the incidence and severity of the PYF phenomenon.

The impacts of PYF factor(s) on yeast fermentation performance and metabolite profile were investigated using mini fermentations (120 ml). The experiments, conducted under stirred and unstirred conditions using high gravity (15°P) PYF+ and PYF- worts originating from the same barley variety, harvest year and region of production, were performed in order to see if any effects consistent with the antimicrobial peptide hypothesis (Axcell *et al.*, 2000) could be found. The results obtained suggested that 15°P fermentations with worts inducing PYF did not have a significant effect on yeast physiological characteristics (i.e. cell density, viability, budding index), metabolite uptake (i.e. sugars, FAN) or fermentation performance (i.e. CO_2, alcohol). Thus, it was suggested that the PYF+ sample used in these experiments was inducing PYF through the presence of 'bridging' polysaccharide mechanism rather than through the presence of antimicrobial peptides. Besides that, it was shown that by keeping yeast in suspension, by mechanical agitation, fermentation progression was quicker and cell density, viability, alcohol yield and CO_2 evolution were higher. Similar trends were also observed in 500 ml brewing fermentations conducted with *Saccharomyces cerevisiae* NCYC 1324 under continuous stirring (Boswell et al., 2002).

The Biolog Phenotype MicroArray system was used for the metabolic characterisation of varying degrees of flocculence yeast strains incubated in different fermentation media (i.e. PYF+ and PYF- worts). The results obtained suggested that the amount of IFY used to stabilise the signal during the analysis, has a significant as well as a direct effect on Biolog reactions. More specifically, it was shown that by increasing the amount of IFY the signal remained stable, even after 20 h of incubation (i.e. the point where the maximum average redox values were observed). However, increasing the amount of IFY (i.e. > 40%) besides increasing the overall cost of the analysis resulted also in "wrong estimates". Besides that, it was also concluded that whilst a lager yeast strain might be insensitive to PYF factor(s) both in small- and industrial- scale fermentations, the same yeast strain may also be sensitive to some other attributes of the same wort (e.g. vitamins, trace elements) during the Biolog analysis. Thus, it was concluded that wort composition has a significant effect not only on yeast flocculation and fermentation performance (Axcell, 2003) but also on the redox dye reduction used to monitor metabolic activity in the Biolog system.

3. Future Work

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 lager yeast strain (e.g. SMA), 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 (e.g. by using microarrays so as to detect potential differences in the expression of the *FLO* genes during PYF+ and PYF- fermentations) in commercially relevant lager brewing strains should help to explain some apparent inconsistencies observed in the incidence of this phenomenon.

In addition, the investigation of the impacts of PYF factor(s) on fermentation and metabolite profiles using the same lager yeast strain and a series of PYF+ and PYF- samples, belonging to the same barley variety, harvest year and region of production and known to have caused PYF both in industrial and small-scale fermentations, could further help towards the elucidation of the antimicrobial peptide hypothesis.

Since supplementation of 6 $mg.l^{-1}$ of linoleic acid and the use of the flocculent lager yeast SMA had a statistically significant impact of yeast flocculation, and therefore on the ability of our in-house PYF assay to distinguish between PYF+ and PYF- worts, the use of an unsaturated fatty acid (e.g. 18:0) would also be an interesting and promising experiment.

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