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Factors that promote Premature Yeast Flocculation (PYF) condition in malt

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Contents

Abstract

Aims

The overall aims of this project were to investigate potential links between malting procedures and the phenomenon of premature flocculation of yeast (PYF) during subsequent brewing. In this preliminary study, commercial malts were tested for the presence or absence of PYF factor and results correlated with production parameters such as barley batch, malting plant or specific malting processes. The intention was to establish what part of the malting procedure has greatest effect on PYF in order to prevent or at least minimise occurrence of PYF in commercial malts.

Conclusions

Tests on more than forty commercial malts have shown that formation of PYF+ve malts in the malting process is reassuringly uncommon. Presence of PYF factor does not appear to correlate with the variety or bulk batch of the barley used, or the malting plant in which it was made, but seems to be restricted to certain operating conditions that are not standard. There are no simple solutions to this complex problem, it cannot yet be statistically linked with any particular process and some established dogma has been invalidated.

Implications

Clearly further work needs to be done to establish the nature of the microbial community through the malting process, and how this community changes with modifications to the malting process.

Introduction

Brewers all over the world, especially but not exclusively lager brewers, experience Premature Yeast Flocculation (PYF) occasionally. Flocculation of the yeast cells at the end of fermentation is desirable but if it occurs before the fermentation is complete (premature flocculation) the flavour of the beer is impaired and the alcohol yield is lower than expected from the extract value of the malt. Malt-induced PYF behaviour has been recently reviewed by Lake and Speers (2008) and has different technical definitions throughout the world. The details vary, but all define PYF as an event that results in the rapid decline of yeast cells in suspension, with or without a high final extract level. The occurrence of PYF in a brewery causes major problems as the beer may require blending to achieve desired product specifications. In extreme cases, if the beer is unusable, disposal costs will be incurred.

PYF can be influenced by a variety of yeast-related factors, but is often caused by malt carrying PYF factor. Research into the nature of the PYF factor suggests that it is a polysaccharide component from the barley husk (Herrera and Axcell (1991), however its presence in malt is not well understood as PYF+ve barley can make clean or PYF+ve malt. It has also been reported that non-PYF barley can make either clean or PYF+ve malt depending on which steep vessels were used (Axcell *et al 1986)*. PYF factor is resistant to thermal processing during brewing and does not decline with storage time, so a brewer has very few options for dealing with a PYF+ve malt, although it can be diluted with clean malt to a level which does not cause PYF in brewing. The aim of this project was to attempt to correlate the presence of PYF factor in UK malts with the malt production conditions, especially during steeping, so that where possible such conditions can be avoided and thus production of PYF+ve malts reduced.

PYF determination is made by monitoring small scale fermentations and measuring yeast in suspension and time taken to flocculate under standard operating conditions. Extracts of malt samples are compared with extracts of known PYF+ve and non-PYF malts. Malt samples were supplied by several maltsters from a variety of sites and production units to investigate which aspect had most influence on expression of the PYF factor in the final malt. These samples were augmented with two experimental malts, produced in the Campden BRI malting plant in an experiment designed to investigate the effects of CO_2 build up during processing.

Materials & Methods

Yeast Propagation

A PYF-sensitive yeast (BRYC 907) was selected from the yeast collection housed at Campden BRI. Yeast cultures were stored in liquid nitrogen and inoculated into 30ml sterile MYGP broth containing glucose, 27.5g/l; maltose, 115g/l; maltose extract, 3g/l; yeast extract, 3g/l and peptone 5g/l. The cultures were incubated for 24h at 25°C whilst shaking, orbitally, at 150rpm. The 30 ml culture was then inoculated into 500ml sterile MYGP broth and incubated for 28h at 20°C whilst shaking, orbitally, at 150rpm. After 28h the yeast cell count was established using a yeast cell NucleoCounter (YC-100, Chemometic A/S, Gydevang 43, DK-3450 Allerød, Denmark) to ensure that cell density was greater than 120 million cells/ml.

Wort preparation

Two controls were included in each fermentation run, a non-PYF malt (negative control) and a PYF +ve malt (positive control). Approximately 53g of test malt or 50:50 w/w mix of test barley and non-PYF malt were ground on a coarse mill-setting having a 1mm gap between mill discs. A 50g aliquot of grist was mashed by adding 200ml deionised water at 45°C and stirring for 30 minutes. After this time the temperature was ramped at 1°C/min to 70°C and a further 200ml of deionised water was added to the mix. The mash was held, stirring, at 70°C for 60 minutes. At the end of mashing the final weight of the mash was made up to 450g and the hot extract filtered through mash filter papers. The first 50ml of the liquid filtrate was tipped back in to the filter. Filtered extract was boiled gently in 1litre Erlenmeyer flasks containing glass beads for 60 minutes. The hot extract (wort) was then filtered through filter paper, cooled to 20°C and gravity was measured after thorough mixing. The volume was adjusted with deionised water to give a gravity of 11°P±0.2°P. The wort was sterilised by steaming for 30 minutes in 250ml aliquots in 1litre Duran bottles and stored at 4°C for no longer than 2 weeks.

Pitching and Fermentation

The yeast cells were harvested by centrifugation at approximately 3000g for 5 minutes and added to 250ml aliquots of wort at a pitching rate of 20 x 10^6 cells/ml. The Duran bottle was shaken 35 times to achieve an approximate 8ppm concentration of dissolved oxygen. After 5 minutes the pitched wort was mixed gently to re-suspend the yeast and divided into 100ml aliquots in pre-sterilised glass separation funnels.

Each funnel was incubated at 12°C±0.5°C for 96h until flocculation occurred. Light transmission measurements were made through the fermentation vessels and were recorded using a custom-built light transmitter capable of holding 20 vessels at one time. This was linked to a computer for data logging. Cell counts were measured after 3 and 4 days incubation using the NucleoCounter. At the end of the fermentation process the data were downloaded to Microsoft Excel and examined graphically for evidence of flocculation in comparison to the positive and negative controls. A positive PYF result was given if the ratio of viable cells in the test sample compared to the negative control samples was less than 0.5 on the 72h count.

Pilot Plant Malt production

Malting barley (variety Tipple) was malted under two sets of conditions (Tables 1-3). The control was malted under optimum conditions whilst the test batch was malted using a reduced flow of fresh air. In each experiment 50kg of barley was steeped using a 1:4.5 barley to water ratio.

Table 1 Steeping conditions for pilot malts

	Steeping Program	% Water Fill	Water Fill Temp (°C)	Set Wet Period (h)	Actual Wet Period (h)	Aeration on (min)	Aeration off (min)	Set air rest (h)	Actual air rest (h)	CO_2 Fan on (min)	CO_2 Fan off (min)	CO_2 Fan Speed (%)
Control	1st Water	100	16.0	8.0	8.0	20	20	16.0	16.0	30	60	35
	2nd Water	100	16.0	8.0	8.0	20	20	2.0	2.0	60	30	45
	3rd Water	0	0	0.0	0.0	20	20	0.0	0.0	60	0	60
Test	1st Water	100	15.0	8.0	8.0	0	0	16.0	16.0	0	0	35
	2nd Water	100	15.0	8.0	8.0	0	0	2.0	2.0	0	0	45
	3rd Water	0	0	0.0	0.0	20	20	0.0	0.0	0	0	60

Table 2 Germination conditions for pilot malts

	Germination Program	Set Duration (h)	Actual Duration (h)	Air On Temp (°C)	Grain Bed Temp (°C)	Fan Speed	Turning Revs	Turning Interval (h)
Control	Stage 1	24.0	24.0	17.5	19.0	60	3	8.0
	Stage 2	24.0	24.0	16.5	18.0	60	3	8.0
	Stage 3	24.0	24.0	15.5	17.0	60	3	8.0
	Stage 4	24.0	28.7	14.5	16.0	60	3	8.0
Test	Stage 1	24.0	24.0	17.5	19.0	40	3	8.0
	Stage 2	24.0	24.0	16.5	18.0	60	3	8.0
	Stage 3	24.0	24.0	15.5	17.0	60	3	8.0
	Stage 4	24.0	28.7	14.5	16.0	60	3	8.0

Table 3 Kilning conditions for pilot malts

	Kilning Program	Set Duration (h)	Actual Duration (h)	Initial air on (°C)	Final air on (°C)	Air off Trip	Initial fan speed	Final fan speed
Control	Stage 1	3.0	3.0	30.0	60.0		100	100
	Stage 2	6.0	6.0	60.0	65.0		100	75
	Stage 3	Until set temp.	1.1	65.0	65.0	32.0	75	75
	Stage 4	10.0	10.0	65.0	85.0		60	40
	Stage 5	2.0	2.0	85.0	85.0		40	60
Test	Stage 1	3.0	3.0	30.0	60.0		100	100
	Stage 2	6.0	6.0	60.0	65.0		100	75
	Stage 3	Until set temp.	3.0	65.0	65.0	32.0	75	75
	Stage 4	10.0	10.0	65.0	85.0		60	40
	Stage 5	2.0	2.0	85.0	85.0		40	60

Commercial Malt production

Three malting companies provided 48 commercial malts from seven different maltings, five British, one Irish and one Belgian, using a variety of different steeping and germination vessels. The malts had been prepared from at least eight different barley varieties for use in both ale and lager breweries and distilleries. Detailed production conditions were provided for some malting protocols. In some cases samples of the original barleys were also available and barleys were also analysed for presence of PYF factor.

Results

Pilot Plant Malting

It is understood by maltsters that maintaining aerobic conditions through the malting process is essential for the prevention of PYF positive malts. This can be achieved by frequent aeration whilst steeping and effective CO_2 extraction during air rests and germination. This experiment was designed to demonstrate that sub-optimal malt processing conditions, where CO_2 build up occurred, would lead to the formation of PYF positive malts. Pilot malts were processed in two batches. The control batch was aerated during steeping and had effective CO_2 extraction during air rests. The test batch was not aerated during steeping, the CO_2 extraction fans were not used and there was 80% recirculation of stale air during germination. The test batch and control batch were matched in all other aspects and both were kilned in a deep bed using typical lager malt conditions. The analysis of the malts produced, shown in Table 4, shows them to be good quality lager malts, typical of those produced in the Campden BRI pilot malting, with no significant difference in standard malt quality parameters. Both would be expected to ferment satisfactorily during brewing.

Table 4 Malt quality of Pilot Plant Malts

	Malt with 100% Fresh Air	Malt with 80% Recirculated Air
Moisture (%)	4.3	4.9
EBC extract 0.2mm(%)	81.6	82.4
Colour (EBC)	3.4	2.9
TSN (%)	0.74	0.74
TN (%)	1.74	1.80
Kolbach Index (%)	43	41
FAN (%)	0.15	0.15
Fermentability	73	72
Wort Viscosity (mPas)	1.54	1.49
Friability (%)	82	87

Figure 1 Fermentation data for 2 malts prepared from same barley stock in the pilot maltings, showing the effect of reduced air.

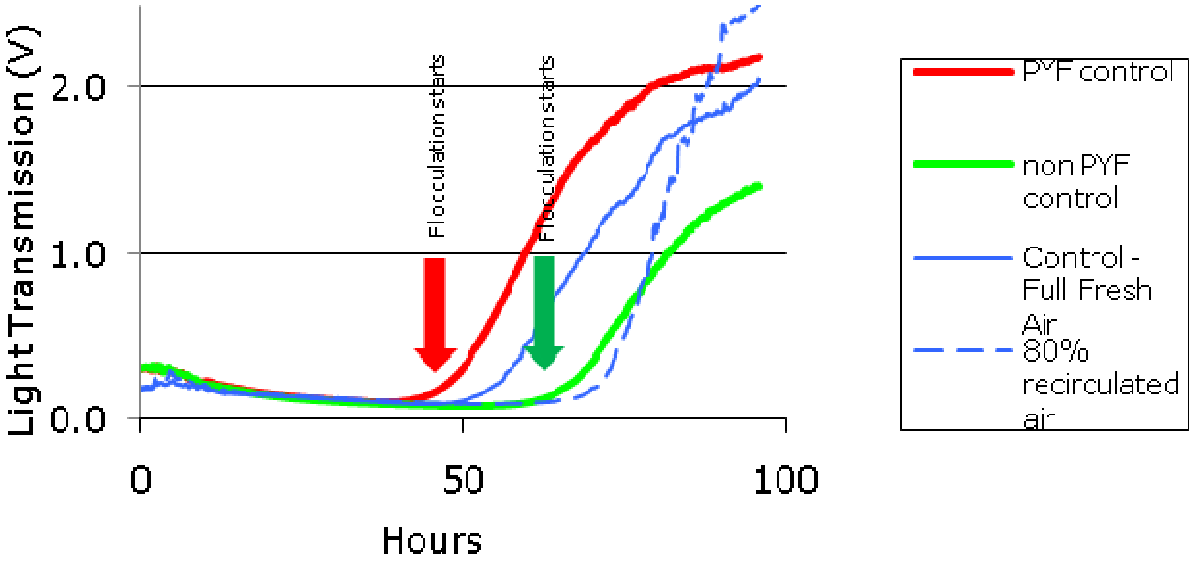
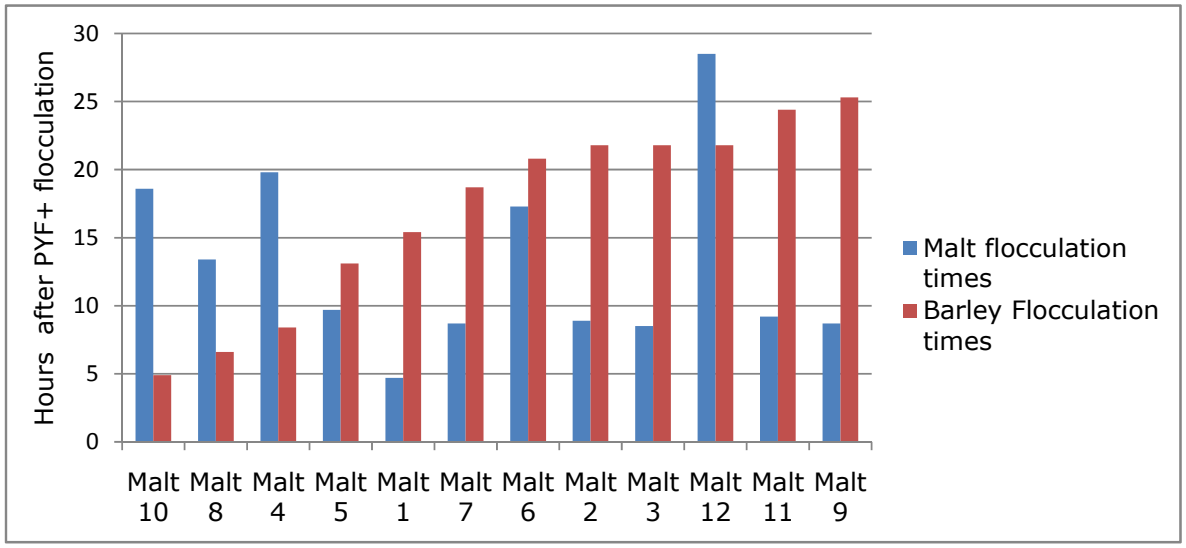


Figure 1 shows that in contrast to all expectations, the barley batch that was subjected to sub-optimal conditions where higher CO_2 built up was PYF-negative, whereas the batch processed normally was PYF-positive. It could be speculated that in the aerobic conditions a particular microbial community was able to flourish and as a result of the microbial activity the PYF factors were formed although this has not been proven in this experiment. Manipulating malting conditions by the addition of antimicrobials has been shown to change the composition of the microbial community with a knock on effect on malt quality (Laitila *et al*, 2007). In their experiments application of antibacterial or antifungal treatments allowed the competitive flora to flourish. It is possible that in this experiment the application of CO_2, which is antimicrobial in high concentrations, prevented growth of the existing microflora. This observation agrees with that of Axcell *et al.*(1986) who demonstrated that when CO_2 increased above 10% v/v it had an inhibitory effect on the microbial population.

Barley Samples

Barleys used in the production of twelve of the commercial malts were analysed for PYF, using a 50:50 mix of test barley and non-PYF malt, as described in the Methods section. Six batches were grown in East Anglia including Pearl and Maris Otter; six were grown in Yorkshire including Flagon; Cocktail and Tipple were grown in both regions. Comparative times of flocculation after that of the PYF+ve control are shown in Figure 2, the shorter the interval between flocculation of the PYF+ve control and the test sample the greater the degree of PYF.

Figure 2 Flocculation times of malts compared with the starting barley.



Malts 2 and 3 were made from the same barley stock and show similar performance, otherwise there is no consistency between the malts and the barleys they were prepared from. In most pairs (75%) the malt exhibited a greater degree of PYF than the barley but in 4 cases (25%), the barley showed more PYF than the malt, indicating that flocculation performance of the grain had been improved by the malting process.

Commercial Malt Samples

In total 48 samples of malt from the 2007 harvest were collected from maltings across the United Kingdom. Of the 48 samples tested only 5 samples were found to be seriously PYF-positive and a similar number were weakly PYF-positive. Table 5 shows the flocculation results and selected anonymous malting data.

Table 5 Results of PYF tests on Commercial Malts

Sample No.	Variety	Barley Moist. %	Barley Source	Final Product Type	Maltings Steep Vessel	Maltings Germn'tn. Vessel	Air Flow rate m^3	Malt Moist. %	Hrs Post +PYF	PYF ratio
1	Pearl	14.7	England	Lager/ale	Conical	Saladin Box	498	4.1	-7.1	0.10
2	Flagon	16.3	England	Lager/ale	Conical	Saladin Box	398	3.5	-4.9	0.10
3	Tipple	14.4	UK	Lager/ale	Conical	Fixed Floor	537	3.7	5.9	0.24
4			UK	Distilling					6.2	0.24
5	Pearl	14.7	England	Lager/ale	Conical	Saladin Box	498	4	6.3	0.30
6	Prestige	14.5	UK	Lager	Conical	Fixed Floor	814	4.8	2.1	0.31
7			UK	Distilling					6.4	0.34
8	Scarlett	14.4	France	Lager	Conical	Fixed Floor	769	4.5	7.8	0.34
9	Scarlett	14.4	France	Lager	Conical	Fixed Floor	779	4.4	9.9	0.34
10	Scarlett	14.4	France	Lager	Conical	Fixed Floor	767	4.6	9.5	0.37
11	Flagon	14	England	Lager/ale	Conical	Saladin Box	449	2.8	3.4	0.40
12	Tipple	15 - 18	England	Distilling	Conical	Round Saladin	≤680	3.9	13.4	0.40
13	Sebast-ian	15 - 20	Ireland			Saladin box	380	4	16	0.40
14	Pearl	14.8	UK	Lager/ale	Conical	Fixed Floor	546	4.2	7.6	0.42
15	Tipple	14.3	UK	Lager/ale	Conical	Fixed Floor	553	4.2	9	0.46
16	Scarlett	12.8	France	Lager	Conical	Fixed Floor	764	4.5	11.8	0.51
17	Scarlett	12.8	France	Lager	Conical	Fixed Floor	765	4.3	12	0.55
18			UK						7.9	0.56
19	Pearl	14.3	England	Lager	Conical	Saladin Box	680	4.8	4.7	0.58
20	Cocktail	15 - 18	England	Distilling	Conical	Round Saladin	≤680	4	8.7	0.60
21			UK	Distilling					13.3	0.65
22	Tipple	14.3	UK	Lager/ale	Conical	Fixed Floor	555	3.7	13	0.66
23	Prestige	14.5	UK	Lager	Conical	Fixed Floor	768	5.2	10.8	0.66
24	Tipple	14.3	UK	Lager/ale	Conical	Fixed Floor	536	4.4	11.1	0.72
25	Flagon	15 - 18	England	Lager	Conical	Round Saladin	≤680	5.4	8.7	0.74
26			Belgium						16.8	0.74
27	Cocktail	15 - 18	England	Distilling	Conical	Round Saladin	≤680	4.6	9.2	0.81
28			UK						17.8	0.81

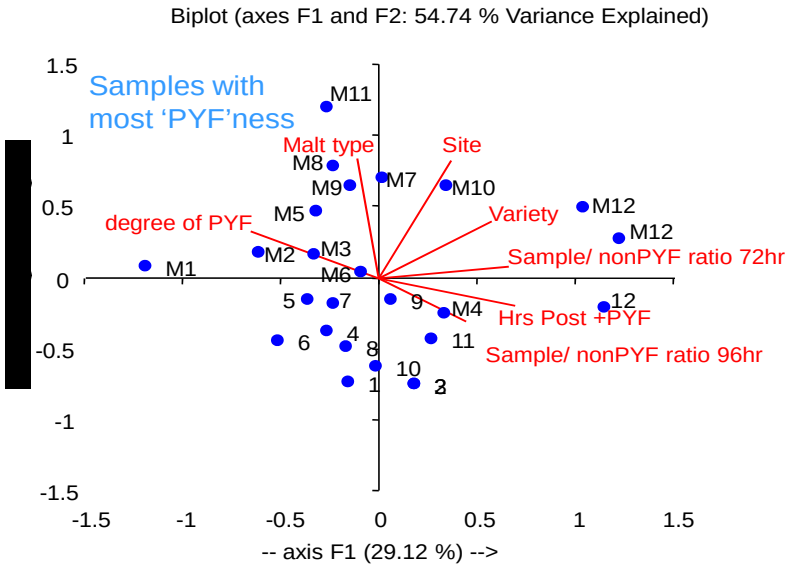
29			UK						12.1	0.81
30	Tipple	14.3	UK	Lager/ale	Conical	Fixed Floor	552	4.4	12.8	0.85
31			UK						13.2	0.87
32			Belgium						17.1	0.88
33	Scarlett	14.4	France	Lager	Conical	Fixed Floor	765	4.4	14.5	0.89
34	Tipple	15 - 18	England	Distilling	Conical	Round Saladin	≤680	4	18.6	0.90
35	Prestige	15 - 19	Ireland			Saladin box	380	4.1	19.9	0.90
36	M. Otter	15	England	Ale	Conical	Saladin Box	680	3.5	8.9	0.91
37			UK						12	0.98
38	M. Otter	15	England	Extra Pale Ale	Conical	Saladin Box	680	4.4	8.5	1.00
39	Cocktail	14.7	England	Ale	Conical	Saladin Box	680	4.1	9.7	1.00
40	Tipple	14.3	UK	Lager/ale	Conical	Fixed Floor	535	4.7	15.4	1.05
41			Belgium						6.6	1.08
42			Belgium						17.4	1.09
43	Tipple	11.7	England	Lager	Conical	Saladin Box	680	5.4	19.8	1.40
44	Pearl	14.1	England	Ale	Conical	Saladin Box	680	4	17.3	1.49
45			Belgium						11.7	1.57
46	Prestige	14 - 20	Ireland			Saladin box	380	3.7	19	1.77
47	Tipple	14.3	UK	Lager/ale	Conical	Fixed Floor	536	4.4	20.6	1.97
48a	Flagon	15 - 18	England	Lager	Conical	Round Saladin		4	25.5	3.82
48b	Flagon	15 - 18	England	Lager	Conical	Round Saladin		4	28.5	4.50

The samples showing highest levels of PYF appear at the top of the table; samples which flocculate less than 5 hours after the PYF+ve control are PYF+ve and if the cell count ratio between a sample and its corresponding non-PYF control is less than 0.5 this also indicates a PYF tendency.

One sample, selected at random, was analysed on two separate occasions to check reproducibility; the results are displayed in Table 5 as 48a and 48b and in Figure 3 as M12. The sample was picked for the reproducibility check before the results indicated that this sample was the least likely malt to cause PYF, flocculating more than 24 hours later than the positive control in both runs and having the highest cell count ratios.

Principle component analysis of 12 samples from a single company with 2 sites in England (See Figure 3) showed no influence of barley variety or of field location on PYF-status. Other published studies on the occurrence of PYF malts in commercial malting found that there was no clear effect of the harvesting location, but did find that certain maltings were more likely to produce PYF-positive malts (Jibiki *et al.*, 2006).

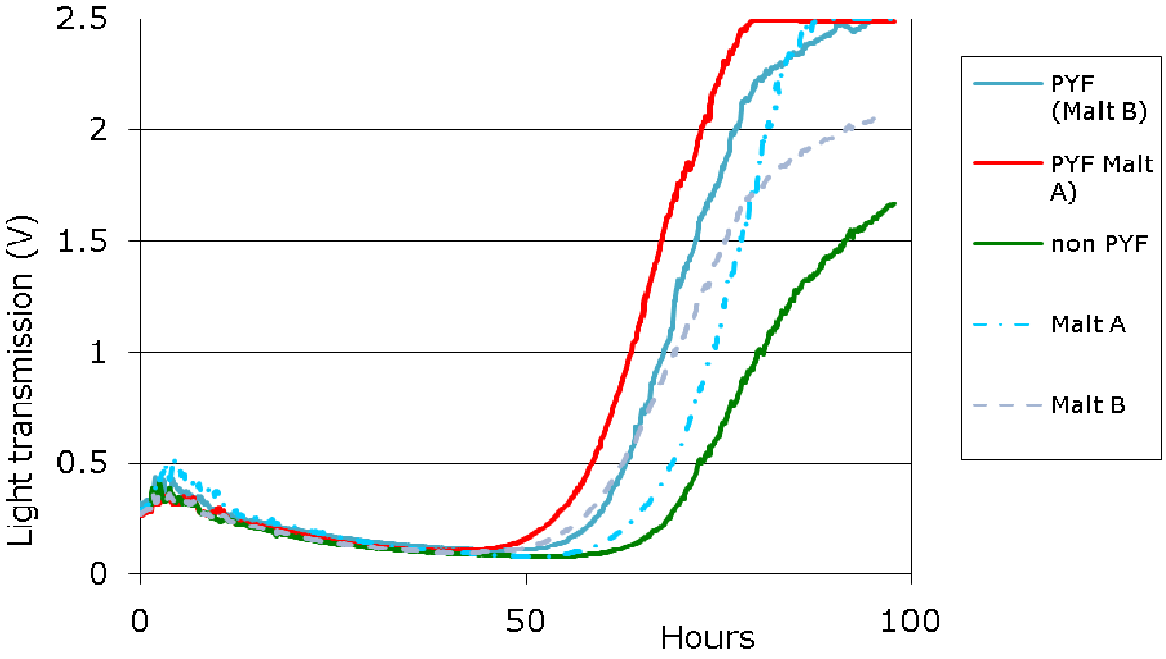
Figure 3 Principle Component Analysis of samples from one Maltster.



Samples labelled by number only are barleys, samples labelled M are malts, M12 was measured twice.

In this study one bulk sample of barley (Prestige) was shown to produce PYF-positive and PYF-negative malts at the same location. The two malt samples shown (A and B in Figure 4) were analysed on different occasions because at the time of testing it was not known that the two samples were related; they appear in Table 5 as malts 6 and 23.

Figure 4 PYF data for 2 malts prepared from same barley stock in the same maltings.



Malt 23 was designated PYF-negative because the cell count ratio of test malt to the negative control at 72h was > 0.5, however when the yeast flocculated there was a steep decline in viable cell number in the fermentation suggesting that some of the PYF factor may have been present but not in a concentration that was high enough to cause early flocculation. Malt 6 was highly PYF-positive. The processing data indicated that this malt got very hot during the steeping process and had high CO_2 levels during the air rests. This provides evidence that high CO_2 during malting can promote the PYF condition but appears to contradict pilot plant data from our own malting plant.

Discussion

Analysis of the available data has not provided any correlations between PYF+ve malts and either their source barleys or their production histories looking at site and process method variation. One site deliberately submitted malts known to exhibit PYF in an attempt to discover the reason; as these are not typical of their usual product quality, on this basis we cannot extrapolate the frequency of PYF occurrence across all the sites or companies involved. Given this lack of obvious correlation, a much larger sample set with complete processing data would be necessary to extract more subtle correlations.

Comparison of malts 6 and 23 show that it is possible that when CO_2 levels are slightly raised it promotes microbial growth or induces a stress response in the barley grain. If CO_2 levels become excessive then these responses are inhibited.
The microflora associated with specific malting plants might have more influence in producing PYF-positive malts than the process control. Recent terminal restriction fragment length polymorphism (T-RFLP) data has shown that PYF-positive malts are frequently associated with particular microorganisms but not all of the microbes implicated have been identified (Kaur *et al., 2008)*. This changes the focus of the investigation away from the malting process itself toward the local microflora and invites further research.

Acknowledgements

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