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The study of premature yeast flocculation and its relationship with gushing of beer

The production of good quality beer requires a consistent performance of fermentation. Many brewers around the world have problems associated with certain batches of malt that experience so-called premature flocculation of yeast. Premature yeast flocculation (PYF) is the phenomenon whereby fermenting yeast coagulates and settles prematurely. Premature yeast flocculation results in a sudden ending of the fermentation process, even if fermentable sugars are still present in the solution. This phenomenon causes an undesirable beer flavour and a low attenuation which results in a reduction of the sugar-alcohol conversion.

This work looks at the prediction of premature yeast flocculation right of barley grain using so-called PYF method and compares the results with the conventional method for the determination of fermentability. Also the results of the PYF method were compared with the results for gushing determination in malt.

It was found that premature yeast flocculation closely correlates with the gushing of beer. Further it was ascertained that the results of the PYF prediction obtained directly from barley grain correlated very well with the results of the conventional method for the determination of fermentability. In practice is possible to substitute for the conventional method.

Descriptors: Spring barley, malt, premature yeast flocculation (PYF), fermentability, barley antifungal activity, gushing

1 Introduction

The production of good quality beer requires, among other things, consistent performance of the fermentation processes. Many brewers around the world have problems associated with certain batches of malt that suffer from heavy premature yeast flocculation (PYF), i.e. the yeast coagulates and prematurely settles at the bottom of the fermenting tub, leaving the fermentation process incomplete and fermentable sugars in solution (Nakamura 1998). Premature yeast flocculation results in an undesirable beer flavour and in a low attenuation, i.e. the sugar-alcohol conversion is reduced. This phenomenon causes sizeable financial losses to breweries (Axcell et al. 2000, Nakamura et al. 1997).

Research into premature yeast flocculation inducing factors, which are present in malt and probably present already in the barley grain, has been going on for a number of years and is carried out in many eminent world-class breweries. The definition of these factors (structure, properties, action effects, origin and localisation) is not nearly resolved, especially on the basis of the brewing raw material complexity, the yeast strains complexity and the complexity of brewing procedures, which can vary in different breweries (Van Nierop 2004).

It has been supposed for a long time that the premature yeast flocculation-inducing factor (PYF factor) is a substance present in the malt which is released into the wort during mashing (Axcell et al. 2000, Nakamura et al. 1997). Recent works point to the presence of this factor already in the barley grain. The PYF inducing factor is presumably also produced partially during the malting process (Axcell et al. 2000, Nakamura 1998).

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Tables and Figures see Appendix.

A number of substances have previously been implicated as compounds causing early flocculation of yeast (Kudo 1958, Kudo, Kijima 1960, Kudo 1959, Morimoto et al. 1975, Fujii, Horie 1975, Fujino, Yoshida 1976, Yoshida et al.1979, Herrera, Axcell 1991). Most of these have been carbohydrate based or contained carbohydrate (saccharide fraction complex composed mainly of glucoarabinoxylans). On hydrolysis, glucose, xylose and arabinose were released often in the presence of uronic acid residues (Axcell et al. 2000).

The sporadic occurrence of the premature yeast flocculation problem suggests that microbial organisms may be involved (Yoshida et al. 1979, Axcell et al. 1986). In the case of pathogenic microorganism impacting on the plant, a host-pathogen interaction occurs. This penetration into the plant organism (often only contact between the pathogenic microorganism and the cell) induces a host of co-ordinated intracellular processes. Their effect is to restrict or completely eliminate the factor leverage and the spread into the other cells. This defensive plant reaction is accompanied by the creation of new substances or by the increasing the content of compounds that are already present (Procházka et al. 1998). Attack by the pathogen results in the induction of number of metabolic changes in the plant. Individual defensive reactions of the plant include the output of specific proteins, the synthesis and the accumulation of chemically simple compounds with distinctive antibiotic effects (Procházka et al. 1998). A special group of defensive proteins is formed of thionins which appear in the cell wall a very short time after contact with the pathogen. They affect a wide spectrum of pathogens, supposedly by the disruption of the cytoplasmic membrane (Bohlmann 1994). They can be bound to polysaccharides containing chitin and β-1,3-glucane, which are components of the cell wall of the fungus (Oita et al. 2000). Thionins occur in the endosperm, roots and germs. They are toxic for a wide range of microorganisms. It has been established that they bind themselves to the yeast by electrostatic interactions with negatively charged membrane phospholipids and then interact specifically with certain structures in the membrane or form pores. After this membrane interaction, thionins induce many relatively rapid reactions in the yeast cells, comprising increased intake of calcium ions, outflow of the potassium ions, alkalinization of the medium and changes of the membrane potential (Thevissen et al. 1996).

Also the formation of lipid transfer proteins (LTP) is initiated. These proteins show hard antimicrobial activity. They are present in epidermal cells and vessel tissues. In addition to the lipid transfer into the organelles, LTPs are also required for the formation of the strengthened cuticle (Axcell et al. 2000).

Initial work concerning the toxic substance (lethal for some brewery yeast) from wheat and barley grain was published in 1970 by Okada et al. Axcell et al. (2000) also identified the protein fraction lethal for yeast. They expressed the hypothesis that perhaps this protein enters into the class of stress proteins, so-called antimicrobial peptides (AMP). These are produced as a response to the microbial growth partly during barley cultivation, partly during the steeping process, and they exhibit the antifungal activity. Antimicrobial peptides are stable enough to survive the brewery procedures and therefore during the fermentation they are able to inhibit the yeast growth by preventing the uptake of sugar, and they can cause the yeast to flocculate prematurely (Axcell et al. 2000). From the aforementioned flocculating yeast a polysaccharide was also isolated. It contained uronic or ferulic acid residue. This acidic polysaccharide, stated as a PYF inducing factor by the Kirin brewery in 1975 (Fujii, Horie 1975), can bind itself nonspecifically to the AMP. The identification of the AMP is then very difficult in these cases, owing to the low relative molecular weight of AMP and to the high relative molecular weight of the polysaccharides. However it is possible that the polysaccharide causes the reticulation (neting) of polysaccharides and yeast, and in this way large flakes could be formed and consequently the premature yeast flocculation could occurs (Axcell et al. 2000).

Herrera et al. (Herrera, Axcell 1991) isolated and characterised the polysaccharide fraction from the malt husks which, according to their results, brings about premature yeast flocculation. The malt husk extract, obtained using the mild water extraction, induced the premature yeast flocculation after its addition to the normal wort and subsequent fermentation. Using SDS-PAGE electrophoresis of this extract, four groups of proteins were identified (42 600, 17 500, 15 100, 13 100 Da) and a polysaccharide of a high relative molecular weight (more than 100 000 Da) was observed. These components were separated using gel filtration (Sepha-dex G100). By means of subsequent experiments (Herrera, Axcell 1991) it was found that only the polysaccharide component induces the premature yeast flocculation. Using the saccharide analysis (paper chromatography) it was found that arabinose, galactose, glucose, mannose, xylose, rhamnose and the acidic saccharide component were all present (Herrera, Axcell 1991). Again it was the acidic saccharide to which antimicrobial peptides can bind.

The formation of substances, arising after microscopic filamentous fungi (micromycetes) have had an impact on the barley grain, is probably in the interrelationship with the occurrence of so-called primary gushing of the beer (Sypecká et al. 2003). Gushing is an undesirable phenomenon occurring, in particular, in bottled beer. It means that, after opening the bottle, a specific volume of beer spontaneously over-foams (Bellmer 1996, Draeger 1996). However, the real gushing inducing substances are still not known. It is expected that these substances are produced as a response to the previous stress of the organism, either during the growth period or during consequent processing of raw materials under certain conditions (microbial impact, technological processing of the barley and the like). The gushing can be caused either by metabolites excreted by pathogens, or by compounds which are released from impaired cell walls of both organisms and they relate to their defence ability. These substances can be, for example, of protein character or they can belong to the polysaccharides (Axcell et al. 2000, Pellaud 2002).

Nonspecific lipid transfer proteins and thionins, which belong to the array of antimicrobial peptides which are supposed to induce the premature yeast flocculation, are strongly cationic with an amphipathic character, and therefore they are capable of producing or stabilizing the foam. And hence it is possible to conclude that gushing appears as a result of the leverage of the antimicrobial peptide produced by barley as a response to the infection. Accordingly this theory presumes the potential mechanism of gushing is causes by microbial contamination (Axcell et al. 2000).

However the whole complex mechanism of reactions, which is triggered (activated) during the pathogen attack of the plant, is very complicated (Procházka 1998). For this reason also the whole issue of the "originators" of the gushing and the premature yeast flocculation is not still satisfactorily resolved. Following on from the above discussion, the gushing and the premature yeast flocculation could be induced by identical factors. Thus in this work the PYF method was carried out in parallel with the gushing determination in malt and the purpose was to ascertain whether the premature yeast flocculation is related to the inclination of malt or barley to gushing, and what is the extent of this relationship.

2 Materials and methods

2.1 Field trials

Field trials were laid down in plots at the Agricultural Research Institute Kroměříž Ltd. (Váňová et al. 2004). Sugar beet and cereal were the previous crops. To achieve sufficient disease severity, the plots were artifically inoculated with spores of F. graminearum and F. culmorum. Their ratio in the inoculum was 1:1. To avoid the leaves being infected by other pathogens, all plots were treated with a combination of Atlas or Cerelux (0.2 or 0.6 l/ha). Fungicides against FHB (Fusarium Head Blight) were applied at the beginning of the spring barley anthesis; DC 61-64 (Decimal Code for the Growth, Tottman et al. 1987). As carrier 0.1 l of Silwet L-77 diluted with 150 l water per ha was added to fungicides. Artificial inoculation with a suspension of fusarial conidia was carried out 24 hours later.

Grain samples were taken from four replications, screened on a 2.5 mm sieve, and ground. 50 g of the samples were used to measure PYF. Screened samples of 2 kg were used for malting.

2.2 Labelling of samples

Vegetation harvested in the year of monitoring (2003) lodged under the influence of a local heavy storm which resulted in grains with a high contamination with fusarium fungi. Names of assigned barley varieties, information about the previous crop, infection and treatment with fungicides and the numbers attached to the individual barley samples are cited in Table 1.

2.3 Malting technology

Barley samples were micromalted, according to the adopted technology for the production of malts, subjected to the gushing test, i.e. 2 days steeping, 3-5 hours in water, 6 days germination and kilning 1 x 22 hours at the temperature of 80 °C for 4 hours.

2.4 Brewery yeast

Brewery yeast was supplied by VÚPS Prague, denomination Saccharomyces cerevisiae var. carlsbergensis, brewery yeast of

bottom fermenting, RIBM 665/127, origin: VLB (Versuchs- und Lehranstalt fur Brauerei) in Berlin, SMA Nr. 333. The yeast was always used fresh and was the same for all the comparative analysis of malt.

2.5 Conventional method for the determination of fermentability

The method gives further information about the malt quality from the viewpoint of the fermentation process in operating conditions. It is based on the fermentation of laboratory prepared wort in special fermenting tubes at 8 °C over eight days by certain tribe of yeast (Basařová et al. 1992). On the eighth day the differences between the fermentation of the sample under test and the standard malt sample are evaluated and the analysed malt is classified in the relevant class of fermentability (Basařová et al. 1992). The method includes mashing the ground malt sample and the standard malt sample, filtration of the hot sweetwort, hop boiling, filtration of the wort, preparation of pitching yeast, fermentation of the wort – performed in duplicate (addition of brewery yeast to the wort, incubation at 8 °C), assessment of the specific gravity of fermented wort using the densitometer (after defoaming) on the eighth day of the incubation.

Simultaneously determination of attainable attenuation (DSP) was also carried out. The yeast (0.5 grams) was added to 50 ml volume of wort. The flask was closed by fermentation tubule embedded in the rubber plug; wort with a given amount of yeast was agitated and left to ferment at laboratory temperature for 72 hours. After the three days standing the wort was filtrated and the specific gravity was determined using the densitometer in the filtrate.

The specific gravity of the standard fermented wort and tested sample measured using the densitometer after 192 hours of fermentation was expressed in Plato degrees [°P]. The Plato degree conveys the content of apparent extract (in original wort or fermenting solution) expressed as a percent by weight. The specific gravity was converted into the Plato [°P] degrees using the given tables (Basařová et al. 1992). According to the difference between specific gravities of fermented worts d [°P] and according to the attainable attenuation DSP (%) the fermentability class was established, as shown in the Table 2.

Apparatuses and equipment

Densitometer with thermostat (DMA 55, Austria)

Laboratory mill (Miag, Germany)

Mash bath with mash beakers and stirrers (CUBE, Czech Republic)

Other equipment, chemicals and procedures are described in detail in the work Blechová (2004).

2.6 The PYF Method

This fermentation method, developed in the Japanese brewery Kirin (Nakamura 1998), was devised for the prediction of premature yeast flocculation directly from the barley grain. It is based on the fermentation of a laboratory prepared nutrient medium with the additive of the barley extract in the special fermenting tubes at 20 °C during two days using a certain tribe of yeast. On the third day the differences between the optical density of the sample under test and the standard barley sample which induces premature yeast flocculation. Then the malt made from the tested barley is evaluated as to whether it will show a tendency to premature yeast flocculation (Nakamura 1998).

This "Kirin" method includes the preparation of the barley extract from 50 grams of the standard barley sample and the preparation of the standard barley sample inducing PYF (mashing of the ground barley sample with addition of the enzyme mixture – Ultraflo, BAN, Neutrase a βamylasy, filtration of the barley mash, volatilization of filtrate to 100 ml, precipitation of the filtrate by ethanol, dissolution of the precipitate in hot water, centrifugation), fermentation test – is performed in duplicate (the preparation of the synthetic medium, addition of the barley extract to the medium, addition of brewery yeast, incubation at 20 °C, blank preparation – addition of water to the synthetic medium), the measurement of optical density of the fermenting medium at 800 nm after the 48 hours incubation (after defoaming).

Evaluation of the premature yeast flocculation-inducing activity (PYF activity) of the barley extract by "Degree of PYF" is performed as follows. For "Degree of PYF" determination an average of optical densities OD_{800} after 48 hours incubation is calculated from the duplicates of one sample ($\overline{OD_{800}}$)(Nakamura 1998):

$$Degree\ of\ PYF = \overline{OD}_{800}\ (blank) - \overline{OD}_{800}\ (sample\ or\ standard)$$

The "Degree of PYF" of the sample barley is compared with that of the control PYF-inducing barley (Fig. 1) (Nakamura 1998).

The tendency of barley to cause premature yeast flocculation is evaluated in accordance with the Table 3.

Apparatuses and equipment

Spectrophotometer (CARY 1E, Australia)

Laboratory disc mill (Super Jolly SJ 500, France)

Mash bath with mash beakers and stirrers (CUBE, Czech Republic)

Heater (Ceran 500, Germany)

Centrifuge (6K15, Sigma, USA)

Other equipment, chemicals and procedures are described in detail in the work Blechová (2004).

2.7 Gushing

A three-day test Carlsberg (Vaag et al. 1993) was used for the determination of gushing in malt. The method is based on the assumption that markers of over-foaming are soluble in water, active and soluble after boiling and active under the conditions prevailing in beer. The method includes the replacement of 50 ml of non-over-foaming finished beer with the malt extract and then three-days of shaking under the defined conditions. The volume of the foamed-up beer after the consequent bottle opening expresses the gushing value (ml).

Apparatuses and equipment

Rotatory mixer (MR 25, MLW, Germany)

Heater (Ceran 500, Germany)

Centrifuge (6K15, Sigma, USA)

Water bath (EI-20R, Kavalier Votice, Czech Republic)

Shaker (P-01, EPO, Frenštát p. R., Czech Republic)

Other equipment, chemicals and procedures are described in detail in the work Blechová (2004).

2.8 Statistical processing

The results were processed statistically using correlation and regression analysis and analysis of variance.

3 Results and discussion

3.1 Relationship between the results of the PYF, fermentability and gushing in malt methods

Long since it was supposed that the premature yeast flocculation is related to the occurrence of pathogen microorganisms on the barley grain. Similarly, as was the case for the emergence of beer over-foaming or primary gushing. Thus both phenomena were studied simultaneously in an endeavour to find out if there was any connection between the acquired results.

For barley samples the PYF group was determined. For malt samples produced from these barley samples the fermentabiltity class and the gushing in malt were assessed. The results obtained are summarized in Table 4 and in the Figure 2 A, B.

The measured results, demonstrated in the Figure 2 A, B, are arranged descending order according to the size of the PYF degree percents of the control PYF-inducing barley for the given samples. Barley was classified into a PYF group according to section 2.6. In cases where marginal values of the percents of degree of the PYF of the control PYF-inducing barley intervene in two groups of barley, then the appropriate group of barley is marked "+" (better), if the range of degree of the PYF of the control PYF-inducing barley also comprises (except given barley group) a the group marked by lower number. The barley group is marked "–" (worse), if the range of degree of PYF of the control PYF-inducing barley comprises (except given barley group) also a group marked by higher number. In the Figure 2 A, B the numerical value 3.75 belongs to the barley group IV+; the numerical value 3.25 to the barley group III– and 1.75 to II+.

Optical density (OD800) was measured, for the majority of assessment, after 48 hours of incubation also after 24 and 72 hours of fermentation. It was found, that the difference in the optical density (OD800) of individual samples is highest after 48 hours of fermentation (Figure 3), which is consistent with previous literature (Nakamura 1998).

Based on the results it was ascertained that the testing of a standard in the PYF method in each determination set is necessary because the degree of PYF for a given sample can diverge in different PYF determinations. This is presumably caused by the high sensitivity to external conditions of the method, for example to the fermentation temperature, to the synthetic medium composition, to the properties of the yeast population and so on. For example also little variations of temperature during fermentation can influence the final value of the PYF degree of any given sample, just as little variations of the synthetic medium composition, which is always freshly prepared for each experiment. Testing of the control PYF-inducing barley is necessary in each experiment in order to compare the results of different assessments. Values of PYF degrees of other tested samples then relate to the value of PYF degree of the control PYF-inducing barley, and then the classification into the PYF groups is carried out (Table 3).

Based on statistical treatment it was found that the results of the PYF method correspond to results of the fermentability determination method. The degree of correlation is rather high, attaining to a value of r = 0.81 (Table 5). The PYF method is therefore applicable to the prediction of premature yeast flocculation straight from the barley grain and it can replace the conventional method of fermentability determination. The PYF method has several advantages against the conventional method. It takes only three days, straight the barley grain is tested and only 50 g of the sample are required for the experiment. In addition, it is possible to test the either dormant or water sensitive barley grains. While the conventional method of fermentability determination is more time-consuming, it takes eight days, a rather large sample is required (320 g) and the malt is tested, not the barley grain. The malting procedure of the barley brings on another time delay (from seven to nine days), and consequential financial loss. In practice the PYF method is therefore more convenient.

Between the results obtained by the method of gushing determination and the results detected by the PYF method, a correlation coefficient of r = 0.59 was established (Table 5). But assuming that the premature yeast flocculation could be caused by the concrete metabolite produced by the barley in consequence of the microbial attack, it is possible to infer from these results that the gushing is caused, also as the PYF, still by some other factors which are not common for these two phenomena. Therefore it can not be really excluded that gushing is caused by the combined effect of several substances of different chemical composition, including PYF-inducing substances. It is possible to suppose the same in the case of the premature yeast flocculation.

3.2 The value of the PYF group, the value of the fermentability class and the gushing value in reliance on the barley variety and the previous crop

The previous crop and the variety influence on the value of the PYF group and the fermentability class was not statistically proved (Tables 6, 7) in the sample collection under review (Figs. 4, 5). The same was observed in the case of the gushing (Fig. 5).

The previous crop and the variety influence on the gushing was studied in detail in the work Havlová et al. (2004, 2005) and it was statistically verified that the gushing value is influenced by the previous crop as well as the barley variety.

Again, a rather high correlation between both methods – the PYF method and the fermentability determination method – was established, both in the case of the cereal as the previous crop (r = 0.84) and in the case of the sugar beet as the previous crop (r = 0.82). Thus it is possible to relate the values of the first variable to the level of values of the second method (Table 8).

A significant affirmative relationship was also established between the gushing in malt and the value of the PYF group, both in the case of sugar beet as the previous crop (r = 0.70) and in the case of cereal as the previous crop (r = 0.72) (Table 8). Again the acquired results acknowledged the assumption that both phenomena are caused in part by common factors.

3.3 The influence of fungicide treatment of barley on the value of the PYF group, fermentability class and gushing in malt

Looking at the results (Figs. 6, 7) it can be seen that barley samples with fungicide treatment exhibit considerably lower PYF groupings (and belong to the better fermentability class) against tested samples without fungicide treatment. That means that the fungicide treatment of barley reduces the value of the PYF group (thus reducing the tendency of barley toward premature yeast flocculation). These results correspond to the hypothesis that the premature yeast flocculation is, among other things, caused by the microbial contamination of barley. In the work Havlová et al. (2005) the influence of fungicides on reducing the gushing

in malt was studied within an enlarged sample set. Differences were found in the efficiency of the fungicides used (Havlová et al., in print). Amistar and Caramba are among the tested the most effective preparations (Fig. 7). Results support the hypothesis that the main cause of primary gushing is the microbial contamination of barley.

3.4 The influence of the artificial infection of barley by fungus Fusarium culmorum on the PYF group value and the gushing

Following on from the results it is observed (Fig. 8) that barley samples, which were artificially infected and then treated with fungicides, show essentially a higher value of the PYF group against other tested samples without artificial infection. It means that the value of the PYF group is increased by artificial infection in spite of the fact that samples were subsequently treated with fungicides. The selected fungicide did not have to be efficient against a specific contamination. The results also show that microfungi presence on the barley grain is related to the occurrence of premature yeast flocculation (PYF). The results correspond to the conclusions of Yoshida et al. (1979) and Axcell et al. (1986).

By comparing the gushing in malt values of artificially infected and non-infected samples (Figure 8), much higher values were found for the infected samples. The same results were obtained in the work Havlová et al. (2005) within an enlarged sample set (Fig. 9). In the year of monitoring, the infection of samples with cereal and sugar beet as the previous crop was much higher. The infection was induced by lodging of vegetation caused by a local heavy storm. In accordance with the higher grain contamination of these samples, high gushing in malt values were also found. Extremely high gushing values in more contaminated samples again affirmed the hypothesis that the gushing is caused by infestation of the grain by pathogen microorganism (Niessen et al. 1992, Bellmer 1996, Dilly et al. 1998, Schwarz et al. 1996, Pellaud 2002). However, the research of gushing still continues.

4 Conclusions

It was found that the PYF method is applicable to the prediction of premature yeast flocculation directly from the barley grain and it can be substituted for the conventional method of fermentability determination. The PYF method can be used in the brewery or malt-house laboratories or in the research institutes.

It is possible to infer from the results that gushing is caused in part by the same factors as the premature yeast flocculation, but also by some other factors. Therefore it can not be really excluded that gushing is caused by the combined effect of several substances of different chemical composition, including PYF-inducing substances. It is possible to suppose the same in the case of the premature yeast flocculation.

The variety and the previous crop influence the value of the PYF group and the value of the fermentability class but this was not statistically proved in the studied sample collection.

Further it was found that the fungicide treatment of barley reduces the tendency of barley towards premature yeast flocculation. These results correspond to the hypothesis that the premature yeast flocculation is among other things caused by the microbial contamination of barley.

Artificial infection of barley increases the value of the PYF group in spite of the fact that samples were subsequently treated with fungicides. Accordingly the results show that microfungi presence on the barley grain is related to the occurrence of premature yeast flocculation (PYF).

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Appendix

Table 1 Labelling of samples

Previous crop sugar beet, artificial infection

Barley number	Barley variety	Fungicide treatment
1	Kompakt	control (without fungicide treatment)
2	Kompakt	Amistar + Duett
3	Kompakt	Folicur BT
4	Kompakt	control (without fungicide treatment)

Previous crop sugar beet, artificial infection, without fungicide treatment		**Previous crop cereal, artificial infection, without fungicide treatment**	
Barley number	Barley variety	Barley number	Barley variety
5	Diplom	20	Prestige
6	Calgary	21	Scarlett
7	Annabel	22	Sabel
8	Respect	23	Amulet
9	Kompakt	24	Nordus
10	Prestige	25	Akcent
11	Olbram	26	Tolar
12	Scarlett	27	Kompakt
13	Sabel	28	Forum
14	Nordus		
15	Amulet		
16	Akcent		
17	Tolar		
18	Jersey		
19	Forum		

Table 2 Determination of Fermentability Class

Fermentability class	*d* [°P]	*DSP* [%]
1	$d < -0{,}3$	–
1	$-0{,}3 < d < +0{,}3$	> 80
2	$-0{,}3 < d < +0{,}3$	$75 < DSP < 80$
3	$-0{,}3 < d < +0{,}3$	< 75
4	$d > +0{,}9$	–
4	$+0{,}3 < d < +0{,}9$	< 73

Note:

d – difference between specific gravities of fermented worts

DSP – attainable attenuation

Table 3 Evaluation of barley tendency to premature yeast flocculation - classification of barley into the PYF group

PYF-inducing barley – group IV Degree of PYF of the sample barley ? 70 % of Degree of PYF of the control PYF-inducing barley
Possible PYF-inducing barley – group III 70 % of Degree of PYF of the control PYF-inducing barley > Degree of PYF of the sample barley ? 50 % of Degree of PYF of the control PYF-inducing barley
Normal barley – groups II + I group II: 50 % of Degree of PYF of the control PYF-inducing barley > Degree of PYF of the sample barley ? 30 % of Degree of PYF of the control PYF-inducing barley group I: 30 % of Degree of PYF of the control PYF-inducing barley > Degree of PYF of the sample barley

Table 4 Results of PYF, fermentability a gushing in malt methods for barley/malt samples number from 1 to 28

Sample	% of Degree of PYF of the standard	PYF barley group	Fermentability class (f)*	Gushing in malt (ml)
1 - standard	100 ± 7	IV	4	138
2	62 ± 5	III	3 to 4	71
3	38 ± 4	II	2	79
4	73 ± 7	IV+	4	74
5	74 ± 11	IV+	2	67
6	59 ± 7	III	2	44
7	43 ± 5	II	2	81
8	69 ± 6	III–	2	25
9	62 ± 5	III	3 to 4	42
10	63 ± 7	III	2	67
11	38 ± 4	II	2	89
12	53 ± 9	III+	2	30
13	81 ± 6	IV	3 to 4	106
14	89 ± 8	IV	4+	42
15	108 ± 11	IV	4	102
16	85 ± 8	IV	4	119
17	94 ± 19	IV	4	119
18	67 ± 7	III–	3	113
19	73 ± 9	IV+	3	130
20	34 ± 6	II+	2	46
21	59 ± 8	III	4+	26
22	39 ± 4	II	3	32
23	73 ± 6	IV+	4	23
24	91 ± 9	IV	4	78
25	133 ± 9	IV	4	129
26	82 ± 9	IV	3	52
27	116 ± 9	IV	4	106
28	36 ± 8	II+	2	17

Note:
* – fermentability class 1 means the best quality of malt from the point of view of brewery process, 4 the worst
Standard – control PYF-inducing barley

Table 5 Relationship between PYF groups, fermentability classes and gushing in malt - correlation analysis (*n* = 26)

	PYF groups	Fermentability classes	Gushing in malt
PYF groups	1.0000	0.8138***	0.5969**
Fermentability classes	0.8138***	1.0000	0.4598*
Gushing in malt	0.5969**	0.4598*	1.0000

Note:
n – number of experiments

Table 6 Analysis of Variance for PYF

Source of Variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. Level
Variety	6.4375	8	0.8047	1.483	0.295
Previous Crop	1.0035	1	1.0035	1.850	0.211
Residual	4.3403	8	0.5425		
Total	11.7813	17			

Table 7 Analysis of Variance for Fermentability

Source of Variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. Level
Variety	8.1319	8	1.0165	2.891	0.077
Previous Crop	0.0000	1	0.0000	0.000	1.000
Residual	2.8125	8	0.3516		
Total	10.9443	17			

Table 8 Relationship between PYF groups, fermentability classes and gushing in malt - correlation analysis (*n* = 9)

Previous Crop: Sugar Beet

	PYF groups	Fermentability classes	Gushing in malt
PYF groups	1.0000	0.8200**	0.6959*
Fermentability classes	0.8200**	1.0000	0.4661 NS
Gushing in malt	0.6959*	0.4661 NS	1.0000

Previous Crop: Cereal

	PYF groups	Fermentability classes	Gushing in malt
PYF groups	1.0000	0.8407**	0.7244*
Fermentability classes	0.8407**	1.0000	0.6530 NS
Gushing in malt	0.7244*	0.6530 NS	1.0000

Note:
n – number of experiments

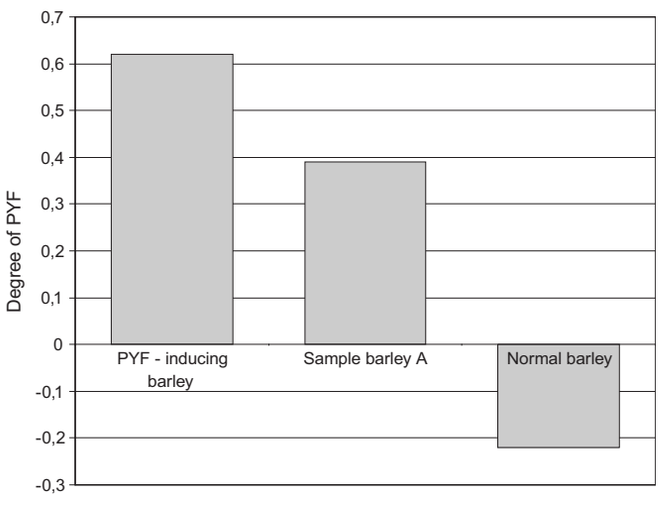

Fig. 1 Example of the result of PYF

The barley sample A was evaluated as being the „possible PYF-inducing barley"; it belongs to the PYF group of value III (70 % of Degree of PYF of the control PYF-inducing barley > Degree of PYF of the barley sample A > 50 % of Degree of PYF of the control PYF-inducing barley).

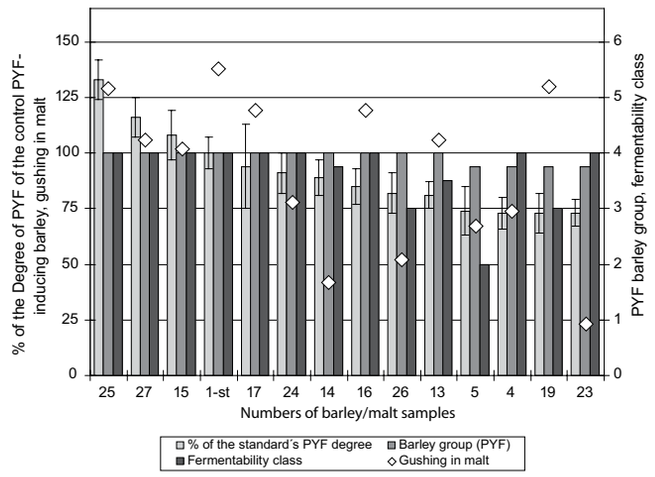

Fig. 2 A Comparison of results of PYF, fermentability and gushing in malt methods - part 1

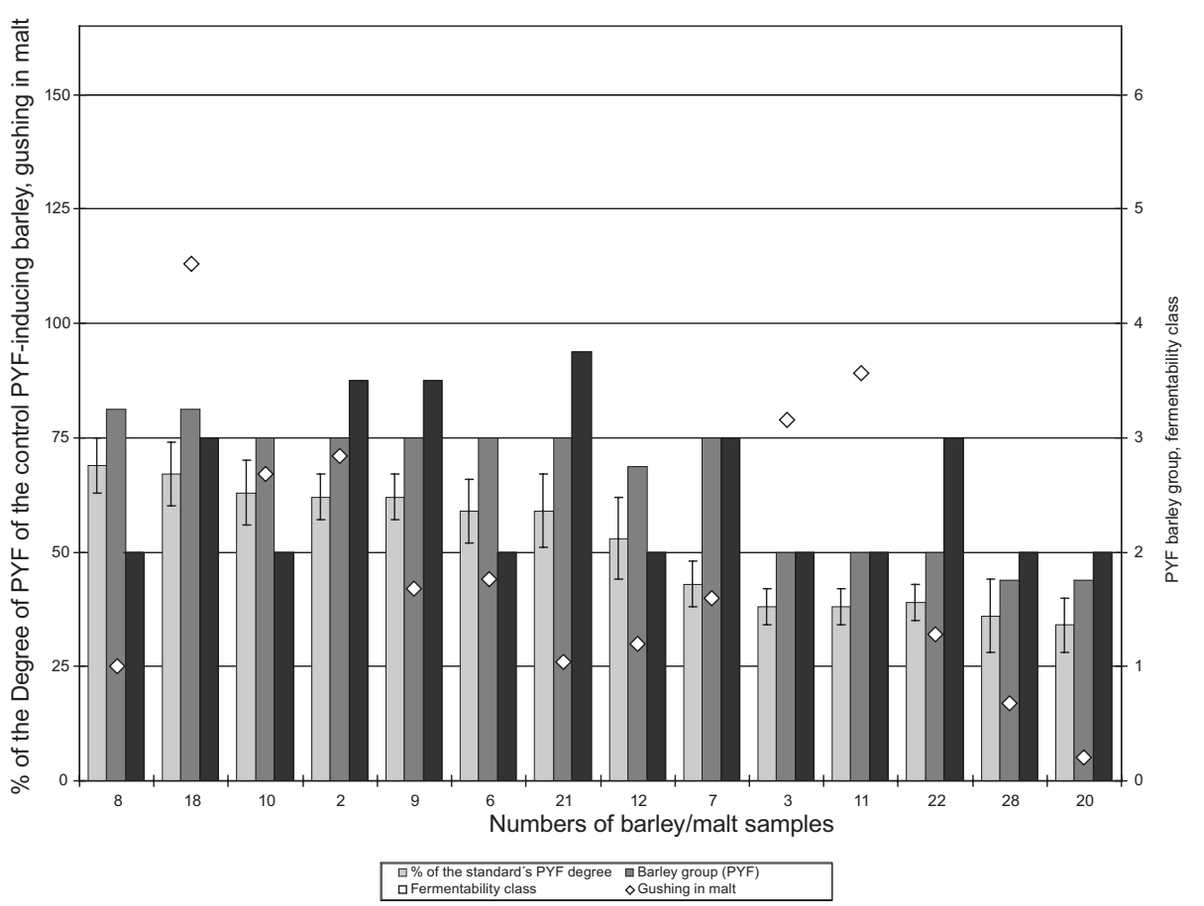

Fig. 2 B Comparison of results of PYF, fermentability and gushing in malt methods - part 2

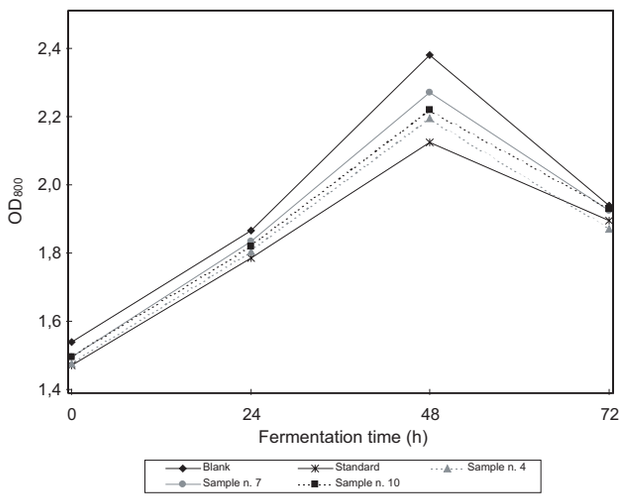

Fig. 3 Typical fermentation patterns of yeast

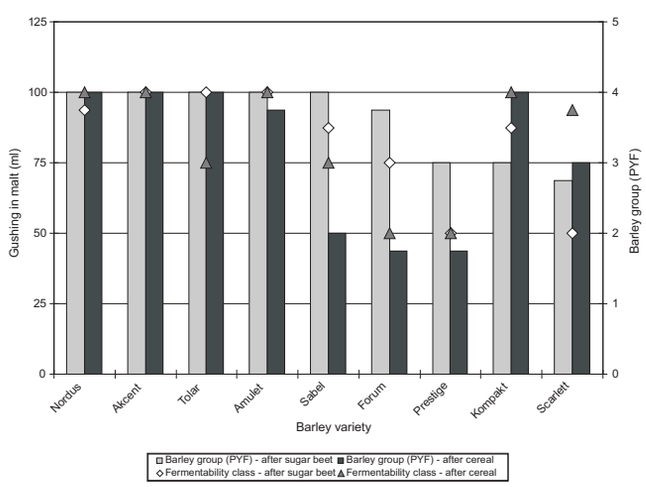

Fig. 4 Comparison of results of PYF and fermentability methods for two different previous crops - sugar beet and cereal

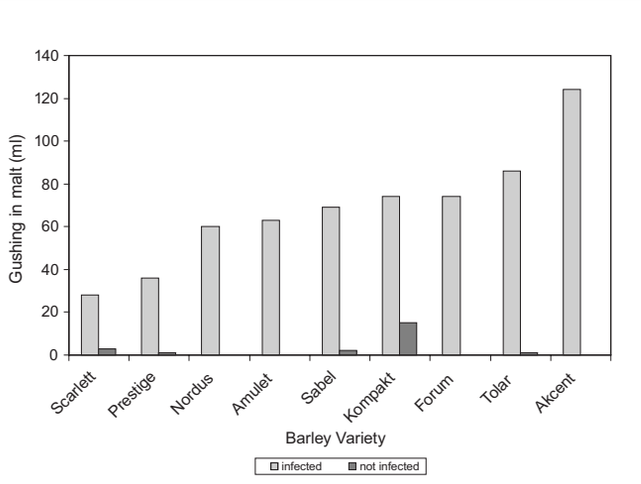

Fig. 5 Comparison of results of PYF and gushing in malt method for two different previous crops – sugar beet and cereal

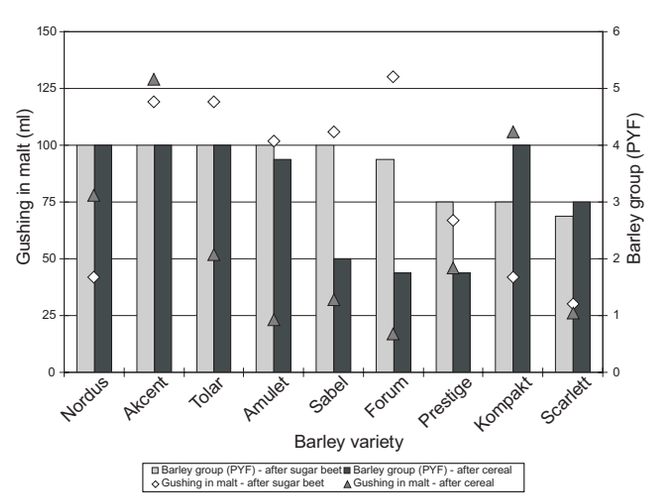

Fig. 6 Comparison of results of PYF, fermentability and gushing in malt methods for the barley variety Kompakt, artificial infection by fungus Fusarium

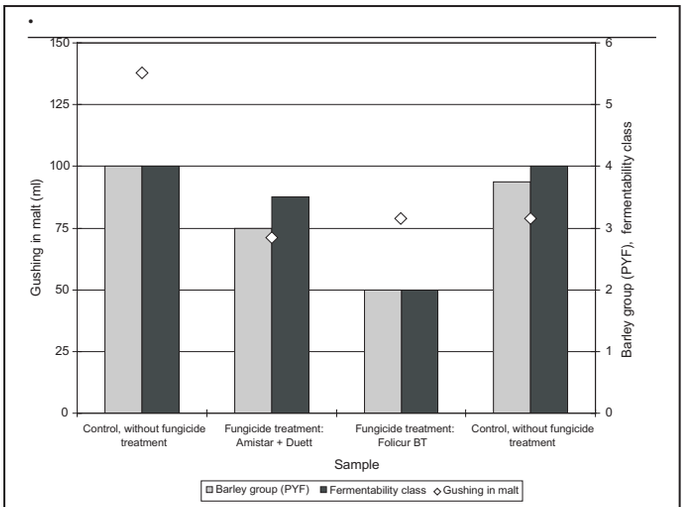

Fig. 7 Comparison of results of PYF and gushing in malt methods for the barley variety Kompakt, artificial infection, previous crop: corn

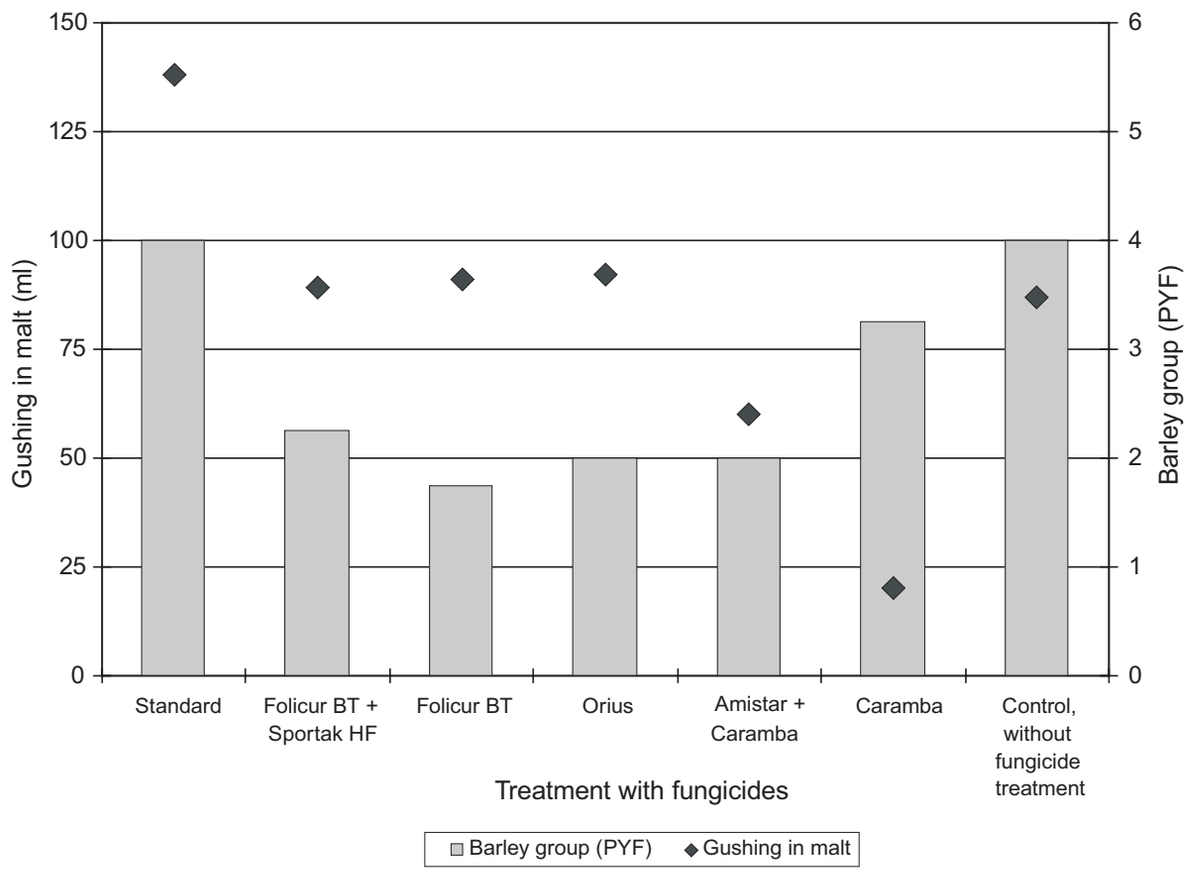

Fig. 8 Comparison of results of PYF and gushing in malt methods, samples without artificial infection and with artificial infection (consecutively treated by fungicides)

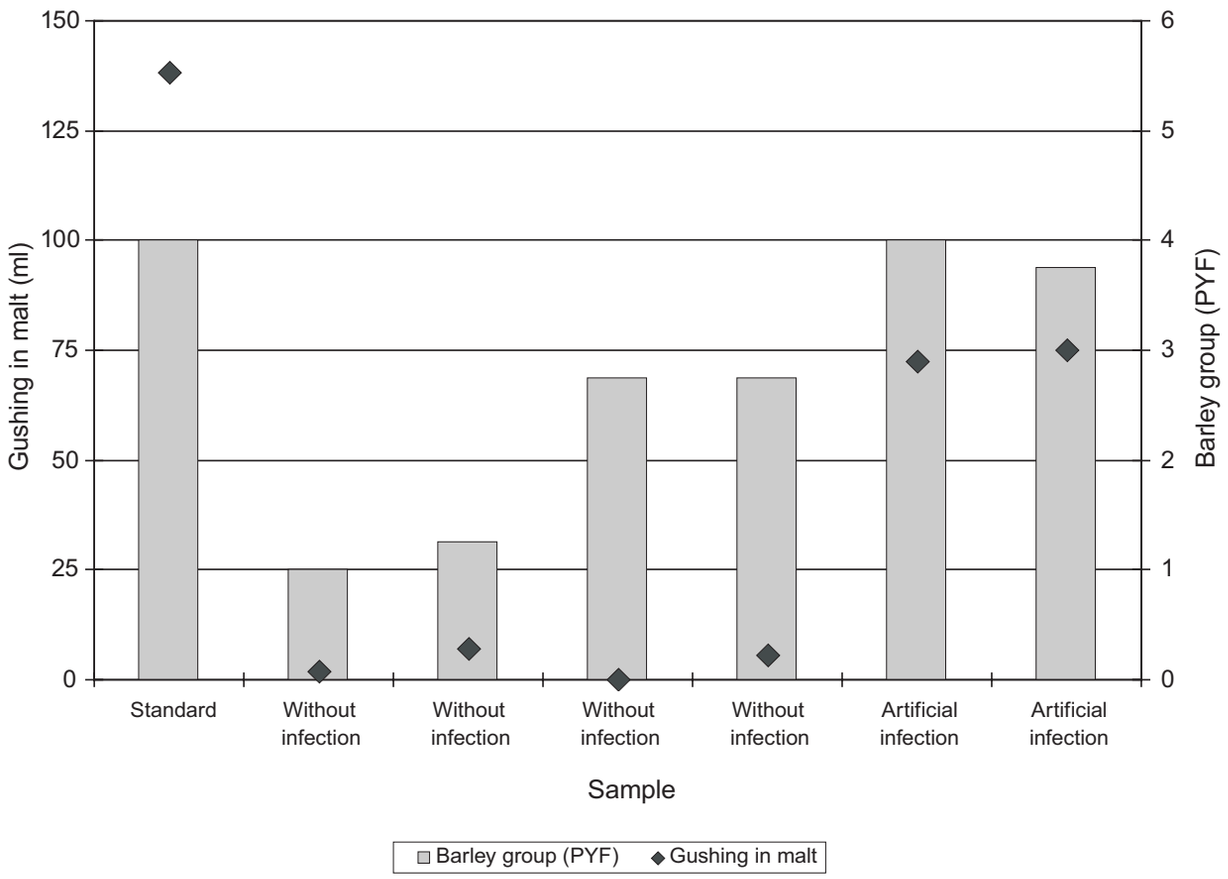

Fig. 9 Comparison of gushing in malt results of artificially infected and not infected samples

Artificial infection: previous crops - sugar beet and cereal

Not infected: previous crop - maize and rape