HGCA

PROJECT REPORT 319

THE ENHANCEMENT OF VALUE IN UK BARLEY CROPS BY LIPID BINDING PROTEINS

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THE ENHANCEMENT OF VALUE IN UK BARLEY CROPS BY LIPID BINDING PROTEINS

by

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1. **Abstract**

1.1 Aims

Lipids can damage the quality of beer. Cereals contain a number of different proteins that can bind lipids, the lipid binding proteins (LBPs). These may offer beer some protection from these damaging effects.

Hence the aims of this project were:
1). To develop a simple method to analyse these proteins.
2). To survey the levels of extractable LBPs in barleys and malts, and to look at the effect of the malting and brewing processes on the resulting levels of LBPs in the final beer.
3). To examine the relationship between LBPs and the microbiological flora on the grain.
4). To examine the relationship between LBPs and flavour in the final beer.

1.2 Conclusions

We now have a simplified method to study and measure LBPs.
Different barleys were shown vary significantly in the level of LBPs that they contained. The levels were not influenced by microbiological loading of the grain, nor by pesticide usage or seed rate.
The malting process influenced LBP activity. The processing aid Gibberellic acid (GA) in particular could cause an increase in extractable LBP. The increases were probably linked to total protein changes. The levels of extractable LBP also increased during kilning.
Most LBP was lost during mashing. LBPs also had an influence on beer flavour so although high levels of LBP enhanced foam and may have promoted flavour stability they also influenced beer flavour.

1.3 Implications

An easier method to analyse LBPs will aid those concerned about foam quality to investigate various stages of the brewing process for problems in this area.

The farmer wishing to add value to the barley crop could select grains with high levels of LBP. Similarly the maltster could also exploit this work to enhance the foaming potential of the malted grain.

The brewer will be able to identify clearly where foam proteins are lost.

2. Summary

2.1 Introduction

Lipids can damage the quality of beer. They are able to destabilize foam and hence give the beer a poor appearance. Lipids can also lead to off-flavours when oxidised. There are however a number of proteins which can bind lipids (LBPs). For example, there are special classes of barley and wheat protein, called lipid transfer proteins, but certain other general non-specific proteins have the same effect. These cereal proteins could have a beneficial effect on beer quality.

2.2 Aims

A deeper understanding of these proteins might allow their characteristics to be exploited more effectively. One particular problem is that these proteins are quite difficult to measure. Hence the aims of this project were:

1). To develop a simple method to analyse these proteins.
2). To survey the levels of extractable LBPs in barleys and malts, and to investigate the effect of the malting and brewing processes on the resulting levels of LBPs in the final beer.
3). To examine the relationship between LBPs and the microbiological flora on the grain.
4). To examine the relationship between LBPs and flavour in the final beer.

2.3 Methods used

An important initial stage of the project was to develop a simple method to analyse LBPs. The standard method involves the use of radioactive lipids, which are expensive and require facilities that are not generally available in most laboratories in the malting and brewing industry. Our method uses the molecular probe ANS, which fluoresces when in a hydrophobic environment. LBP activity was determined from the change in fluorescence when ANS was displaced from LBP by lipid. This approach avoided the problem of changes in fluorescence due to certain naturally occurring materials in the grain.

The LBP BSA showed a strong lipid binding reaction whereas the hydrophilic protein gelatin did not bind and could be used as a negative control.

2.4 Key results

2.4.1 Surveys

Soluble protein extracts from barley and malt show lipid-binding properties using the ANS test. Early experiments seemed to indicate that malt had a slightly higher lipid-binding capacity than barley.
However the amount of extractable (soluble) protein also increased during malting. This effect could lead to confusion because in some cases there was an increase in total LBP and yet when adjusted for the general increase in soluble protein there was an apparent decrease in the amount of LBP. Hence many experiments are reported as total LB capacity per gram of grain.

There was a significant difference in the LBPs levels of different barleys. There was no relationship between LBP and malting grade nor was there a relationship between total nitrogen and LBPs.

There was generally an increase in the total LBP concentration during malting although specific activity did not show much change.

The malting process had a significant influence on LBP activity, however, the increase during germination was variable. Those barleys that had less LBP tended to show the greater increase during malting, thus the LBP capacities of malts were not significantly different. Gibberellic acid stimulated this increase.

Kilning appeared to increase the level of extractable LBP. The reasons for this are discussed in the technical section.

The greatest loss in LBP during the malting and brewing process was during mashing. A fraction of LBP capacity was unstable to heating near 65°C, however, thick mashes did improve stability.

Reports in the literature suggest that LBPs are an important part of the plant's response to fungal attack. There are no reports, however, that indicate if different farming practice with regards to growing conditions or fungal treatments, would change the levels of LBP. There was no clear correlation between LBP levels and the extent of fungal contamination, nor did fungicide application or seed rate influence appear to influence LBP levels.

2.4.2 Tasting

There have been indications in earlier projects that LBPs may influence the flavour of the final beer. In this project, during a triangular aroma test with control beer and beer + LBP (in this case BSA), 15 of 20 tasters correctly identified the modified beer, i.e. a highly significant result.
They identified the modified sample aroma as:
- less hoppy,
- more diacetyl,
- more cereal,
- more off-flavours,
- less hoppy,
- less fruity,
- slightly musty and cardboardy.

Clearly BSA had an appreciable effect on the aroma profile of beer. It appears that BSA "masked" some of the positive aroma notes resulting in the detection of negative aroma notes.

2.5 Conclusions and implications

2.5.1 For the analyst:

We now have a simplified method to study and measure LBPs.

2.5.2 For the farmer:

Barleys vary significantly in the amount of LBPs that they contain. The levels are not influenced by micro-biological loading of the grain, nor by fungicide usage or seed rate.

2.5.3 For the maltster:

The malting process influences LBP activity. GA in particular can cause an increase in extractable LBP. The increases are probably linked to soluble protein changes. Kilning also causes increased levels of extractable LBP.

2.5.4 For the brewer:

Most LBP is lost during mashing. LBPs also have an influence on beer flavour so although high levels of LBP are good for foam and may promote flavour stability they may also have an influence on beer flavour.

3. Technical detail

3.1 Introduction

3.1.1 Background to Lipid Binding Proteins

Lipid binding proteins (LBPs) are a diverse group of low molecular weight proteins that interact with, bind, modify and/or transfer lipids in some way. A subgroup of these are called lipid transfer proteins (LTPs), these also bind lipids but have a specific role in the transfer of fatty acids from one membrane to another. Thus all LTPs may be considered to be LBPs but not visa versa.

Due to their non-polar properties, many lipids do not exist easily at high concentrations within the cytoplasm of a cell. Indeed a high concentration of soluble lipids in the cytoplasm can cause serious biochemical problems for cell stability. A number of different proteins, found in the cytoplasm and extra-cellularly, are known to bind lipids. Thus there are many different types of LBPs with differing lipid-binding affinities and functions. Some LBPs, such as membrane-associated proteins, may be non-specific in their interaction. These proteins may normally be embedded in the lipid part of a membrane bilayer and their interaction with lipid, whilst strong, is not part of their primary function. On the other hand some proteins, for example, Bovine Serum Albumin (BSA) are designed to interact with a broad range of lipids.

Other enzymes that metabolise lipids are also able to bind them and there could be classes of LBP that have not yet been recognised.

3.1.2 LBPs protect plants against fungal invasion

Lipid transfer proteins are especially important in conferring on the plant a natural resistance to fungal attack. A considerable amount is known about barley LTPs as this seems to be the plant system of choice for these studies. LTPs are found in the cell walls of plants (notably in barley leaves) and are formed in response to fungal attack. Moreover, several LTP genes in barley are up-regulated in response to the infection by various strains of fungal pathogens. It is not certain if this protective mechanism is dependent on the ability of the LTP to transfer lipids; clearly, however, the transfer of lipid from a fungal membrane could be damaging (Molina & Garcia-Olmeido, 1993, Garcia-Olmedo *et al.* 1995). This antifungal property of LTPs may well be linked to their interaction, as cationic proteins, with fungal membranes.
LTPs are also amphiphilic (Kader, 1997) and other similar anti-fungal compounds such as Benzethonium Chloride and hop acids have these properties also (unpublished results).

There has not been any study of the relevance of these proteins to fungal resistance during malting despite the importance of fungal micro-flora for malt quality.

LBPs have been located in the endosperm of wheat, oat and barley grain (Ponz *et al.* 1984). They are also known to be protease and amylase inhibitors (Bernhard *et al.* 1989, Jones & Marinac, 1995). Sequence similarities have been shown between barley LTP and that of barley and malt cysteine endoproteinases inhibitor.

3.1.3 LBPs protect beer against lipid damage to foam and flavour

Lipids are very damaging to beer quality. There is very clear evidence that lipid material destabilises the head of foam on a beer. Lipids are also clearly implicated in the formation of trans-2-nonenal that is a major contributor to stale, musty off-flavours in beer. LBPs are able to ameliorate these effects.

Lipids in beer originate from two main sources, they can be extracted from raw materials (malt, hops, yeast) during brewing or they can be introduced at point of sale from foods commonly consumed with beer, such as crisps, peanuts and chips. LBPs are beneficial in dealing with both of these.

3.1.4 LBPs and Beer Foam Stability

The head of foam on a beer is formed from three important ingredients: barley malt proteins, hop bitter acids and carbon dioxide from the fermentation. During bubble formation hydrophobic proteins (derived from the barley) are cross-linked by hop bitter acids forming a semi-rigid structure around the bubble and so stabilising it (Simpson & Hughes, 1994). Lipids interfere with the hydrophobic proteins and prevent a suitable interaction. The result is that the bubble is unstable and the head of foam collapses rapidly (shown in Figure 1, Roberts *et al.* 1994).

The addition of lipid binding proteins to the beer aids recovery of foam stability after lipid challenge (Morris & Hough, 1986; Clark *et al.* 1994). These experiments clearly show that beer to which lipid has been added has very poor foaming qualities but when LBP is added at the same time or before the lipid, the foaming qualities recover very quickly. What is especially interesting is that beers without added LBP also recover but at a very much slower rate (Onishi *et al.* 1995). The implication here is that beers naturally contain LBPs derived from the barley malt, albeit at a lower level than can be achieved by addition. This is in keeping with current ideas on the role of LTP as an important foam protein in beer. Work at BRI has also shown that immobilised LBP can be used to treat beers that have sustained lipid damage (Dickie *et al.* 1998). Our trials have shown that of almost 40 different brands of beer (from all over the world), 19 beers showed an improvement of 20% or more in terms of foaming quality. Some beers showed an improvement of greater than 60%.
This indicates that for a significant number of beers, foam potential has been damaged by lipids during processing, and the problem has been recognised in a number of breweries.

A patent specification from Kunst *et al.* (1997) suggests that LBPs can be added to beer in order to protect and improve foam. Other additives such propylene glycol alginate also improve foam quality by interacting with lipids (Bennet, 1993). Clearly it would be preferable to use the naturally ability of barley proteins to protect the quality of the beer. Nevertheless, very little is know about how malting and brewing procedures influence the behaviour of foam active proteins or anti-foaming lipids.

3.1.5 Beer Flavour and LBPs

The flavour stability of beer is an immensely complex issue. It is not at all clear why there is a flavour difference between fresh beer, mature beer and stale beer. It is clear however that the presence of the compound trans-2-nonenal (as well as other aldehydes) at very low levels (1ppb) is associated with cardboard, musty stale flavours (Devraux *et al.* 1981). These aldehydes are derived from lipids, most probably the unsaturated lipids of barley. The breakdown of these lipids occurs via oxidation by lipoxygenase (Lulai & Barker, 1975, 1976; Baxter, 1982) and leads to the formation of aldehydes. The pathway in between is unclear. Complex metabolic pathways have been identified in living plants but these are unlikely to occur in beer so exactly how oxidised lipids become staling aldehydes is unknown.

LBPs are able to protect lipids from oxidation. As bound lipid is less available than free material oxidative enzymes such as lipoxygenase are unable to use bound material as a substrate (Ek *et al.* 1997).
Much has been made in recent years of the role lipoxygenase in malt quality and beer flavour stability. Most comments have been of an adverse nature. The fact that barley also contains lipid binding proteins that will protect beer foam and flavour stability has been entirely forgotten. This project will redress that balance.

3.1.6 The role of LBPs in Wheat and Barley Quality

Wheat LBPs (puroindoline-a and -b) are now accepted as an important factor in bread-making quality (Dubreil *et al.*, 1998; Koojiman *et al.* 1997). The protein friabilin present in wheat endosperm is also linked to wheat quality and shows considerable homology with puroindoline (Oda & Schofield, 1997). Evidence suggests an inverse correlation between wheat hardness and puroindoline content (Dubreil *et al.*, 1998).
Like beer foams, the formation and stability of dough foams, similar to beer foam, are adversely affected by non-polar lipids (triglycerides and free fatty acids). Puroindolines are thought to have a beneficial influence on dough quality in the same way as LBPs in beer foam (Dubreil *et al.*, 1997; Wilde *et al.* 1993,).

It has long been accepted that a proportion of wheat in the mashing grist is beneficial to beer foam. Part of this benefit is protection against lipid destabilisation and so is likely to stem from an increased availability of LBPs (Morris & Hough, 1987). However, wheat additions to the mash can create problems including difficulties with wort separation, flavour differences and hazes. There could then be advantages in avoiding the use of wheat if the same foam-enhancing benefits could be obtained from malted barley.

3.2 Aims of the Project

LBPs have been isolated from many plant sources including cereals; they have been identified in wheat, barley and barley malt. The very nature of their presence in the raw materials used for malting and brewing make them ideal candidates for further

investigations. Although much is known of the activity *in vitro* of LTPs their true biological role remains unclear.

The influence of LBPs on barley quality is not clearly defined. Several LBPs are present including a friabilin-like protein (Jagtap *et al.* 1993). LTPs are clearly implicated in beer foam quality and could protect beers from flavour damage. The role of LBPs in malting performance and the protection of the grain from fungal attack during malting is not fully understood.

The work described in this project provides a better understanding of:

- The role of LBPs in barley quality.
- The interaction of LBPs with other components during malting and brewing.
- The potential of LBPs for improving the quality of beer.

3.3. Abbreviations

ANS, 1-anilino-8-naphthalenesulphonate; BSA, Bovine serum albumin (an LBP); LA, linoleic acid; LBP, Lipid binding protein; LTP, Lipid transfer protein;

3.4 Materials and Methods

Fatty acid free BSA was obtained from Sigma and used as a model LBP. All beer used in this study was prepared in the BRi pilot brewery.

3.4.1 Origin of the barley samples.
The Agricultural and Dairy Advisory Service (ADAS) provided samples of Optic barley. They were grown as 54 replicated plots using the following in puts:

Seedrate

1 100 seed/m^2
2 400 seed/m^2

Nitrogen

1 50 kg N/ha
2 100 kg N/ha
3 150 kg N/ha

Fungicide

1 Amistar Pro 2l/ha plus
Unix 0.67 kg/ha GS 30-31
2 Opus 1.0l/ha plus
Corbel 0.5l/ha GS 30-31
3 Amistar Pro 2l/ha plus
Unix 0.67 kg/ha GS 30-31 +
Amistar Pro 2l/ha GS 45-59

Other samples were obtained via the BRi malting facility.

3.4.2 Malting protocols.
Unless stated otherwise barley was malted in the BRi mico-maltings using 350g of grain, prepared with a steep/air rest schedule of 7/17/7/17/1 hours. The grin was dried for 8 hours at 45 ^{o}C and 16 hours at 65 ^{o}C.

Larger scale malting experiments were conducted in the BRi pilot maltings as described by Kelly (1987).

3.4.3 Brewing protocols.
Beer was prepared in the BRi Pilot brewery using 50kg of Optic barley malt.
The malt was milled in a two roll mill, and mashed at a liquor to grist ratio of 3:1 at 67 ^{o}C. After 60 minutes the temperature was raised to 78 ^{o}C and the mash was sparged for 60 minutes during wort separation in a lauter. The wort (130 L) was boiled for 90 minutes with 15.5g of $HOPCO_2N$. After cooling the wort was adjusted to 1042 gravity and oxygenated to 18 ppm. The wort was pitched with 6 g/L with yeast and fermented for 6 days at 12 ^{o}C. After fermentation the yeast were removed and the beer was conditioned for 3 days at 3 ^{o}C prior to packaging into bottle.

3.4.5 Microbiological methods.
A sample of 10g of malt was briefly ground in 100ml water using an ultraturax homogeniser. The extract was diluted in a 2-fold series again in sterile water and 100 ul samples were spread on plates of OGYE (Oxytetracyclin, glucose, yeast extract) agar. Plates were maintained at 25 ^{o}C and the number of colonies that could be observed was counted after 7 days. The measurement procedure was the same for both yeast and fungi, the different types were distinguished by colony morphology.

3.4.6 Preparation of protein extracts.
The cereal samples were finely milled with a Buhler Miag mill using a 0.2 mm grind. The milled sample was extracted with a 40 mM acetate buffer pH 5.6 using a 3:1 buffer to sample ratio for 1 hr at room temperature. The sample was centrifuged at 14 k rpm for 15 minutes after which the supernatant was boiled for 30 minutes and then cooled for 1 hr in ice. The solution was centrifuged at 14 k rpm for 15 minutes and the supernatant was carefully removed, the pellet was discarded. Protein in the supernatant was precipitated with saturated ammonium sulphate followed by centrifugation at 14 k rpm for 15 minutes. The supernatant was discarded and the pellet re-suspended in de-ionised water followed by dialysis overnight with de-ionised water (3500 MWCO). The dialysed solution was lyophilised and stored at –20 ^{o}C until required. A second series of samples was prepared in exactly the same way but precipitation was carried out at three ammonium sulphate concentrations, which were 30%, 50% and 80%.

3.4.7 Forced ageing of beer.
Staling compounds can be formed in beer over a period of time, a process that can be replicated in a shorter time by force ageing the beer. Therefore the effect of LBPs on the formation of staling compounds in force aged beer was investigated. Beers spiked with LBPs were aged by a combination of storage at 0°C for 24 hr followed by 50°C for 24 hr, this cycle was repeated for a week. Samples were analysed for aldehyde content by GC-MS.

3.4.8 LOX inhibition assay.
The LOX assay used in this study is a variation of the method devised by Baxter. This measures the formation of hydroperoxides by the change in absorbance at 234 nm using a spectrophotometer. Linoleic acid (50 ul) and protein extract (50 ul) were added to 2.85 ml of phosphate buffer (0.1 M; pH 6.8) in the cuvette in a spectrophotometer. The production of hydroperoxides was measured at 234 nm for 10 minutes, with results being reported as change in absorbance per minute.

3.4.9 Lipid binding assay.
The fluorescence from ANS increases when it is associated with a hydrophobic environment such as a hydrophobic site on a protein. Lipid binding capacity was then determined as the decrease in fluorescence observed when lipid was allowed to displace ANS from the protein. This assay is discussed in more detail within the results section.

Protein dilution series were prepared with 40 mM sodium acetate containing 40 mM sodium phosphate pH 5.6 buffer. To 2 mL of protein solution was added 10 uL of ANS solution (8 mM ANS in 10 mM sodium phosphate pH 7). The mixture was allowed to stand for 30 min at room temperature.

10 uL of linoleic acid (LA) in ethanol (6.25 v/v %) was then added to each tube of one dilution series and the mixtures were allowed to stand for a further 30 min. The samples were then diluted 1:2 with 40mM sodium phosphate buffer containing 40mM sodium acetate, pH 5.6 buffer. The fluorescence was determined using a Perkin Elmer Luminescence Spectrophotometer LS30 at an excitation wavelength of 390nm and a scanned emission spectrum from 410 to 610 nm. Fluorescence measurements were taken of two protein + ANS dilution series samples of which one series had lipid added to them. The maxima in the emission spectra were found. Maximum fluorescence intensity was plotted against concentration for both dilution series (± LA). In each case there was a linear relationship, the slope of which indicated the protein surface hydrophobicity. The difference between the two slopes – the decline in fluorescence when lipid was added to the protein + ANS mixture – was defined as the lipid binding capacity. Experiments were carried out in duplicate.

As expected the amount of extractable (soluble) protein also increased during malting. This effect could lead to confusion because in some cases there was an increase in total LBP and yet when adjusted for the general increase in soluble protein there was an apparent decrease in the level of LBP. Hence in most experiments LBPs activity is reported as total LBP capacity, that is LBP activity measured per unit of protein times the total protein in extract.

3.4.10 Determination of fusel oils and total VDKs.
Samples were placed in glass vials with excess ammonium sulphate, shaken on a rotary shaker for 30-35 minutes and the headspace analysed by GC. For determination of total diacetyl and 2,3-pentanedione the sample was heated in a vial at 60°C for 90 minutes, then shaken on a rotary shaker for 30-35 minutes and the headspace analysed by GC.

3.4.11 Determination of aldehydes in beer.
O-(2,3,4,5,6-pentafluoro-benzyl) hydroxylamine (PFBOA) solution (0.5 mL), 50 uL sodium thiosulphate solution and 50 uL undecylenic aldehyde (internal standard solution) were added to a 5mL aliquot of degassed beer. The solution was mixed vigorously before being placed in a water-bath at 25°C for two hours. After cooling, 50 uL 9M sulphuric acid was added and the solution mixed vigorously for 10 seconds. Hexane (1 mL) was then added and the mixture agitated for a further 30 seconds before the phases were allowed to separate for 30 minutes at ambient temperature. The aqueous layer was removed with a Pasteur pipette. The remaining hexane fraction was washed three times using 5 mL 0.05M sulphuric acid, allowing a minimum of 5 minutes each time for phase separation before removal of the aqueous layer. The hexane phase was then dried over anhydrous sodium sulphate, filtered through cotton wool, and sealed in a crimp-cap vial before analysis by GC-mass spectroscopy.

3.4.12 Protein measurements.
Total protein concentrations of extracts were determined using the bicinchoninic acid method as supplied, in kit form, by Pierce and Warriner (Chester, UK).

3.4.13 Other barley and malt analysis.
The standard analyses for barley and malt presented here were performed according to the Recommended Methods of the Institute of Brewing.

3.5 Results and Discussion

3.5.1 Method development

Lipid binding proteins are difficult to measure. The formal procedure for LTPs involves the transfer of radioactive materials from one lipid body to another. For this reason a simpler method of measurement was sought.

The molecule ANS binds to the hydrophobic region of proteins with a change of fluorescence. In free solution ANS does not fluoresce, when bound in a hydrophobic pocket of a protein, however, the molecule fluoresces (Winkler 1962, Turner and Brad 1968). Unfortunately in complex systems such as grain extracts there can be non-protein molecules that also bind ANS and promote fluorescence (Bamforth *et al.* 2001).

This interference can be overcome by using the assay in a competitive format. In this system the test solution (barley/malt extract, wort beer) is mixed with an excess of ANS and the change in fluorescence is measured. A predetermined quantity of lipid is then added to the mixture. This lipid is able to displace ANS from its bound position in proteins and this is observed as a decrease in fluorescence. Figure 2 shows in diagrammatic representation the principle behind this method. Figure 3 demonstrates the principle using a potent lipid binding protein Bovine Serum Albumin (BSA). This protein contains 12 binding sites for lipid as its biological function is to bind and transport lipid (Curry *et al.* 1998).

Figure 4 compares the lipid binding capacity (by this assay) of BSA with an extract from barley. The change in gradient of the binding curve for barley protein is not as great as that for BSA. This is in no way surprising since BSA was selected for having a very potent lipid binding capacity. Nevertheless the method shows that barley extracts contain lipid-binding capacity.

For the purposes of this work LBP is determined by the change in fluorescence when lipid displaces ANS from a protein mixture. By necessity this will comprise a mixture of proteins. Furthermore the mixture will be composed of both specific LBPs and proteins that are able to bind lipids in a non-specific manner. It may be noted, however, that the hydrophobic proteins implicated in foam formation of beer (Slack and Bamforth 1983) are both specific and non-specific in type. Thus, although this assay is detecting a group of proteins, this group is likely to overlap in a major part with the functionally defined foam proteins. This assay therefore has a functional value. Table 1 compares the lipid binding capacities of selected proteins/extracts with BSA using this method.

Table 1. Lipid binding capacity of selected proteins

Protein	Δ slope ±LA	FA-binding capacity
BSA	54,114	1
Gelatin	0	0
LTP extract	8790	1/6
Soluble barley extract	1906	1/17

Gelatin was selected because it is known to be entirely hydrophilic with no lipid binding capacity (Nakai *et al.* 1996).

A number of methods were examined to see if they would enhance the measurement process. Crude extract was separated in to three fractions using Fast Protein Liquid Chromatography (FPLC) and a G25 sephadex (size separation) column (see Figure 5). The protein fraction could then be separated from smaller material (<5000 mwt). Only the high molecular weight fraction (protein) showed lipid-binding capacity by this method. This procedure was routinely incorporated into the assay method, other clean-up options were also explored but none of these were used later (Table 2).

Table 2. Other clean-up options explored but not used.

Procedure	Effect
Activated charcoal	Protein was lost after treatment
CHAPS non-denaturing detergent	No lipid removal observed
Triton X-100 - detergent	Detergent reacts with ANS
Dialysis against BSA	No lipid removal observed
Passage through LBP column	No lipid removal observed

The final analysis procedure selected is described in the methods section. From this point forth the LBP capacity is defined by the method described.

3.5.2 Survey of barley and malts

3.2.1. Barleys. Figure 6 shows the LBP capacity of a selection of barleys. The range of barleys examined included accepted malting grade as well as feed varieties. There appeared to be a significant difference between the LBP capacities of different barleys (table 3). There was no clear evidence, however, that malting varieties contained either more or less LBP than did other varieties.

Table 3. Significance levels of difference in LBP contents between barleys

	Hanna	Intro	Jewel	Chariot	Fanfare	Halcyon	County	Pewter
Hanna			*		*	**	**	
Intro			*		*	*	*	
Jewel	*	*		*				
Chariot			*		*	**	**	
Fanfare	*	*		*				
Halcyon	**	*		**				
County	**	*		**				
Pewter								

* : $p < 0.05$; ** : $p < 0.01$ Blank indicates no significant difference

3.2.2 Malts. Figure 7 shows the same data as figure 6 but includes the LBP capacities of malts made from the barleys described above. Compared with the data for the barley it is clear that:

1. All of the malts had increased LBP capacities.
2. The variability of the result had also increased
3. The barleys with the lowest LBP capacity seemed to develop more additional capacity than those barleys with higher levels. There appeared to be a convergence of LBP capacity. Statistical analysis (comparable to that of table 3) showed that there was no significant difference between any these malts (for this reason the table has been omitted).

3.5.3 Survey of the Malting Process

Various aspects of the malting process were explored in order to determine their potential effect on LBP capacity.

It has been suggested that a barley with higher nitrogen would contain more LBP. Figure 8 shows the absolute LBP capacity of two samples of Chariot barley with two different nitrogen contents (1.9% and 1.3%). There was no significant difference between these two samples. Thus although a barley with higher nitrogen may contain a higher level of LBP, this is not necessarily the case.

Figure 9 shows the development of total soluble protein and lipid binding protein during the malting process. The development of total soluble protein follows the expected profile with a steady increase from day 1 of germination. The level of extractable LBP showed an earlier increase with a peak at approximately day 1, followed by a decline. By day 4 of germination the level of LBP that could be obtained were only slightly higher than the levels in the original barley. This confirms the observations shown in figure 7 i.e. that the level of LBP in the malt may not be much higher than that in the original barley. It also indicated why the level in the malt was found to be more variable than that in the barley, clearly the level in the malt is in a state of rapid change towards the end of malting. If the germination process is a little quicker or if germination is terminated a little sooner the level may change very significantly. It is also clear from this figure that the LBP content is not closely linked to the total soluble protein of the grain.

Figures 10,11 and 12 illustrate the effect of gibberellic acid (GA) on the development of LBPs during malting. In the cases of both Hanna (grade 2) and Jewel (grade 6) the use of GA resulted in a higher level of LBP in the final malt. In the case of Fanfare (grade 9) there was no significant difference between malts prepared using or not using GA. Gibberellins are natural plant hormones that control the development of some enzymes including the proteases. Added GA had more effect on feed varieties probably because endogenous levels of GA were lower. This different effect with different barley grades could be due to enhanced protease activity stimulated by GA leading to increased formation of soluble protein. Malting grades generally form sufficient proteases whereas feed grades do not.

GA has been added here in order to mimic the effect of different levels of endogenous hormone.
It is understood that some maltings are prohibited from the use of GA and it is not the intention of this experiment to promote the use of GA. Nevertheless this experiment does clearly demonstrate that the malting procedure can influence the final outcome with regards to LBP level.

The level of extractable lipid binding protein increased during kilning. Figure 13 shows that during a lager kilning regime the increase was very small, but an ale kilning regime resulted in a considerable increase in the amount of LBP that could be extracted. It is not

clear why there should be an increase in the LBPs measured at this particular stage. Possible reasons include:

A. Rapid synthesis of LBPs due to heat stress. This would be likely if one or more of the LBPs were a heat shock protein.
B. The increase may not be due to synthesis de novo but a change in conformation of pre-existing protein with heating. This might expose a greater proportion of the hydrophobic component of the protein. This suggestion is in line with work reported in the literature (Bech *et al.* 1995).
C. Heat treatment of the grain may aid release and hence extraction of the LBPs. This would not be consistent with the situation seen during mashing (see below).

3.5.4 The relationship between LBP level and microbiological loading of the grain with different agronomic practice.

It has been reported in the literature that LBPs act as a defence mechanism to fungal attack. Thus grain that has suffered fungal attack may have elevated levels of LBP. Conversely grain that has been effectively treated with fungicides may show low levels of LBP with subsequent problems due to a shortage of foam protein. It was not the intention in this part of the project to investigate the relationship between LBP's and microbiological loading. This aspect has been taken as given. Rather the aim here was to investigate different agronomic practice that might influence LBP levels through a fungal influence. Thus grain was grown using different fungicide applications and also using different seed rates. It is expected that the relevance of fungicide treatment is clear. It was anticipated that different seed rates would provide different susceptibilities to fungal attack. A control, with no fungicide, was not investigated because this would not be representative of genuine farming practice.

This part of the project was carried out in two different ways.

1. A comparison of grains treated with different fungicides (giving a different history of microbiological infection).
2. Analysis of microbiological loading and LBP level on different grain.

3.5.4.1. A comparison of grains treated with different fungicides (giving a different history of microbiological infection). These differences were also investigated at different seed rates. It was anticipated that these grains would have different loading of micro-organisms and a correlation between fungal/yeast loading and LBP content was sought. Figure 14 shows the levels of fungi and LBPs in grains sown at 100 and 400 per square meter when treated with different fungicides. (Seed rate was examined because it was considered possible that the density of plants could influence susceptibility to fungal infection and hence LBP levels.) Figure 15 shows the same data for yeast levels.

The treatment abbreviations are as follows:

Table 4 Fungicide key to Figures 14 and 15

Abbreviation	Treatment
AU	Amistar Pro 2l/ha plus Unix 0.67 kg/ha GS 30-31
OC	Opus 1.0l/ha plus Corbel 0.5l/ha GS 30-31
AUA	Amistar Pro 2l/ha plus Unix 0.67 kg/ha GS 30-31 + Amistar Pro 2l/ha GS 45-59

Although the grain with fungicide treatments did show different levels of fungal and yeast loading there was no clear evidence that was related to the LBP content of the grain.

Figure 16 shows the average levels of LBPs that could be isolated from these grains. There was no evidence that the different fungicide treatments resulted in different levels of LBP, nor that seed rate had any effect on this result.

3.5.4.2. Analysis of microbiological loading and LBP level on different grain.
In a second experimental approach the LBPs and fungal levels in a set of randomly collected grains were examined. Figure 17 shows the level of LBP extracted against the number of fungal colonies isolated.

Once again there was no clear evidence of a relationship between the LBPs content and the number of fungal colonies that could be isolated from the grain.

Conclusion
It had been suggested that LBPs are involved in defense against fungal attack. If that is the case then it is possible that different agronomic practice might influence the levels of LBP in the final grain.

We could not find any evidence that different treatments with fungicide or different seed rates had any influence on the LBP content of the grain.

This aspect of the work was not pursued further.

3.5.5 Survey of the brewing process

Figure 18 shows the levels of LBP that could be obtained during the brewing process, this is compared with the levels of total protein in the same brew.

Table 5 Key to Figure 18

Abbreviation	Stage
Malt	Cold extract of malt
EM	End of mash
SW	Sweet wort
PRB	Pre-boil wort
POB	Boiled wort
PRF	Pre-fermentation
POF	Post-fermentation

Note: Pre-boil wort was been diluted, sweet wort was undiluted.

The cold malt extract was taken as the initial standard and used as a control throughout the experiment. The level of LBP generally followed the changes that are revealed by the total protein. The possible exception to this was during mashing when a substantial decrease was noted with only a small decrease in total soluble protein.

A significant increase in total LBP was observed between sweet wort and boiled wort. This increase mirrors a similar increase during kilning (see above). In the case of kilning three possible reasons for this increase were suggested (increased synthesis, change in conformation, easier extraction). In the case of boiling wort clearly there could be no synthesis and extraction was not an issue. For this reason it is likely that there was a change in the conformation of malt/beer proteins during heating that resulted in them showing increased lipid binding capacity. This observation is in line with published literature (Bech *et al.* 1995) and work with an antibody to foam proteins (Parker D.K. pers. comm.) where increases during heating were noted. This explanation does, however, beg the question 'why does LBP capacity decline during mashing?' Explanations for this include:

A. There may exist different classes of LBP. Overall loss or gain does not reflect the possibility that an entire class of LBP is lost at a particular stage or that a new class is formed by conformational change.
B. There exists a dynamic equilibrium between formation and loss during heating. At moderate temperatures (eg mashing) LBP is predominantly lost by precipitation but the heating effect is not sufficient to form more. During boiling again material is lost but the energy input, being much greater, enhances formation above the rate of loss.
C. LBPs may be particularly sensitive to the protease activity in a mash, there is very little protease activity in during wort boiling.
D. LBPs may be particularly sensitive to special conditions pertaining in a mash (e.g. oxidation, protein cross-linking).

There was a second decline in the level of LBP on pitching with yeast. In this case it was likely that the loss was due to association between protein and the yeast surface. This was mirrored by the decline in total protein at the same time.

The profound loss of LBP during mashing may be considered to be disadvantageous. For this reason some aspects of mashing were explored in order to determine if any mashing condition could preserve a greater level of LBP. Figure 19 shows the influence of mash thickness on the levels of LBP surviving the mash (adjusted for mash thickness). Thicker mashes did preserve the level of surviving LBP and the greatest yield was achieved in mashes with thickness 2 parts liquor to 1 part of malt. The differences were not large but they might be significant when changing a mashing regime for example from lauter to filter systems.

With the exception of a more rapid decline during mashing the changes in total extractable protein were essentially paralleled by the changes in LBP capacity. This suggests that many of the changes in LBP capacity were due to changes in the absolute amount of protein available and were not specific for LBP.

3.5.6 Flavour Binding Studies

3.5.6.1 Preliminary studies with BSA

Initial studies utilised a potent lipid binding protein as a model. Bovine serum albumin was selected for this purpose because it is a potent lipid binding protein available essentially lipid free and in large amounts. When BSA was used in these studies beers could only be assessed for aroma, it was not possible to taste the beers as they had been spiked with a non-food grade LBP (BSA). This meant that the influence of LBP on taste was not studied and some information regarding taste could not be obtained. For example the influence of LBP on mouthfeel or sourness or any changes in hop compounds due to LBPs, could only have been detected upon tasting the beers.

A lightly flavoured lager was spiked with increasing amounts of BSA and the BRi sensory panel was asked to assess the beers for any differences in flavour (aroma only) using a triangular (3 glass) taste format. In this format 2 of the beers were the same and the third (in random order) was different. The tasters were asked to identify which beer they considered to be different. Clearly one third of tasters should identify the different beer correctly only on the basis of random chance. However if a significant number of tasters correctly identified the different beer then there was a significant difference between the beers. The panel were also asked to be as descriptive as possible in their interpretation of any perceived difference between the beers.

In the triangular test a significant proportion of the sensory panel identified the spiked beer, which was described as musty and cardboardy with reduced hop and fruity characters. Since the taste panel had highlighted a significant difference between the control beer and the BSA spiked beer , these beers were subjected to a more detailed analysis to identify the differences in flavour active components. GC headspace analysis has shown that the volatile composition varies when a LBP such as BSA is added to beer (Table 6).

Table 6: Effect of varying concentrations of BSA on the volatile components of beer as measured by GC headspace analysis.

	0 mg/mL BSA	**10 mg/mL BSA**	**20 mg/mL BSA**
Diacetyl	0.06	0.09	0.128
2,3-Pentanedione	0.02	0.03	0.034
Acetaldehyde	9.35	8.85	8.94
Ethyl Acetate	14.50	14.32	14.34
iso-ButylAcetate	0.06	0.06	0.154
n-Propanol	14.33	17.18	21.8
iso-Butanol	14.43	16.88	19.56
iso-Amyl Acetate	1.48	0.88	0.898
iso-Amyl Alcohol	69.43	73.10	75.44
Ethyl Hexanoate	0.13	0.08	0.082
DMS	56.67	62.33	62.6

All as mg/L. The results shown are the averages of 5 analyses.

Compounds such as diacetyl and iso-butyl acetate increased with higher concentrations of BSA, while compounds such as acetaldehyde, iso-amyl acetate and ethyl hexanoate decreased with higher concentrations of BSA.

The BRi sensory panel, who assessed beers spiked with BSA, produced a detailed aroma profile. An aroma profile was generated by attributing a score with respect to the strength of each aroma note, the scores could then be illustrated as a set of spider diagrams (Figures 20,21,22). The spider diagram combined with detailed tasting notes provided a very comprehensive breakdown of the aroma profile of the beer being assessed.

The aroma of the control lager was sweet and alcoholic, with citrus grapefruit notes and a strong fruity ester character that was described as being like tropical fruits. The malt attribute was biscuity and bran-like, whereas the hop character was floral and somewhat spicy. Some DMS was noted on the aroma, as well as a yeasty, bready sulphur quality.

The beer spiked with 10 mg/mL BSA had an increased sweetness, which was probably due to the greater toffee/caramel diacetyl character. The GC data supports this finding as it was found that there was an increase in the concentration of diacetyl in the headspace. The malt character was also increased, and while this was similarly described as biscuity there were also Horlicks/Ovaltine notes. The sample had less worty/grainy aroma than the control, and less DMS. The DMS result was perhaps slightly surprising when considering the GC data where it was shown that the DMS concentration in the headspace increased by 62.6%. Some panelists thought that the aroma had certain negative chemical, cheesy and musty attributes.

The cheesy, rancid negative notes were much more apparent in the beer spiked with 20 mg/mL BSA, as were the synthetic/chemical aromas, which is reflected in the higher scores for solvent attributes. The biscuity character, whilst still evident, was considered to be stale and musty. The sweet, toffee aromas all increased further, while the fruity characters decreased, particularly the citrus notes. The worty/yeasty aromas were higher than in the 10 mg/mL spiked beer, but comparable to those in the control.

Spiking of the control beer appeared to have little effect on the hop characters i.e. hoppy/spicy/floral. However, earlier studies have shown that LBPs can bind hop compounds reducing the intensity of the hop aroma and bitterness in the beer. It would have been interesting to have obtained some HPLC assessment of the hop components in the spiked and control beers and related them to the findings of the sensory panel.

3.5.6.2 Studies with wheat proteins

Wheat LBP extracts were used rather than barley LBPs since quite large quantities were required for tasting studies and the yield of LBPs from wheat was substantially greater than from barley.

The experiments described above were then repeated with the addition of a wheat protein extract to beer. This also had an effect on the volatile components but it was less pronounced than with BSA. This may be due to a smaller concentration of protein being added to the beer, approximately 2 mg/mL compared to 10 mg/mL or 20 mg/mL BSA (Table 7). However, it was also apparent that the specific volatile components affected were different. This can be more easily illustrated when the percentage changes in volatile components are graphed (Figures 23 and 24). For example components such as n-propanol and iso-butanol decreased very slightly when wheat LBPs were added but increased with BSA.

Table 7: Effect of wheat extract on the volatile components of beer as measured by GC headspace analysis.

	Control Beer	**2 mg/mL Wheat**
Diacetyl	0.062	0.062
2,3-Pentanedione	0.02	0.02
Acetaldehyde	5.16	5.35
Ethyl Acetate	10.90	11.14
iso-Butyl Acetate	0.068	0.068
n-Propanol	11.92	11.64
iso-Butanol	16.38	15.82
iso-Amyl Acetate)	0.748	0.714
iso-Amyl Alcohol	72.04	71.60
Ethyl Hexanoate	0.088	0.08
DMS	26.6	30.2

All as mg/L

Further work is required with the addition of higher quantities of wheat proteins to elucidate their role with respect to the binding of flavour and aroma compounds.

3.5.6.3 Inhibition of the formation of trans-2-nonenal

The oxidative degradation of polyunsaturated fatty acids has a role to play in the formation of nonenal and other potential staling aldehydes. This may occur through either or both of two routes, non-enzymic and enzymic oxidation. The enzyme responsible for fatty acid oxidation is lipoxygenase (LOX) which is formed in the embryonic tissue of barley. Although LOX is relatively heat labile there is evidence to suggest that oxidation of fatty acids can occur during mashing (Walker *et al.* 1996) leading to the production of hydroperoxides that can oxidise further to trans-2-nonenal and other aldehydes. The presence of trans-2-nonenal in beer can lead to flavour problems as it has a stale cardboardy taste. It is therefore important to elucidate the influence LBPs may have on the formation of staling compounds.

LBPs could prevent enzymatic oxidation from occurring during mashing by binding the substrate of LOX, the fatty acids. Therefore pure LOX, purified from soya bean, was used together with BSA and protein extracts obtained from wheat and barley to ascertain if LBPs can inhibit LOX. When BSA was added to the LOX enzyme assay a gradual, linear reduction in the rate of reaction of LOX was observed (Figure 25). From the results it can be seen that there was a 60% reduction in the rate of reaction when 10 mg/mL BSA was added to the reaction. When the protein extracts of unmalted wheat and barley were added to the LOX assay, a reduction in the rate of LOX activity was also measured. The reduction was not linear, like that observed for BSA and at high protein concentrations leveled-off. However at low protein concentrations the cereal extracts were far more efficient at inhibiting LOX activity. For example at a protein concentration of 2.5 mg/mL there was a 15% reduction in the rate of reaction due to BSA but a 70% reduction in the rate of reaction with barley malt 913P extract. The malting process also appears to influence the inhibitory effect of the protein extracts in that unmalted barley and wheat do not inhibit LOX as well as the protein extracts from the two barley malts. At 2 mg/mL protein the barley extract had produced a 45% reduction in LOX reaction rate whereas the protein extract from malt 917P had produced a 68% reduction. It was also interesting to note that the degree of modification of the malt had no appreciable effect on the LOX inhibition. For example 913P was a well-modified malt, whereas 917P was an under modified malt, and the results show very little difference in the degree of LOX inhibition between the two.

Note: The designations 913P and 917P refer to two different malts prepared in the BRi Pilot maltings.

These results show that LOX was being inhibited by cereal extracts. It was necessary to attempt to identify the agent that was causing the LOX inhibition therefore barley malt 913P was fractionated further. This was achieved by ammonium sulfate precipitation and resulted in the production of three protein groups referred to as the 30 %, 50 % and 80 %

fractions with respect to their ammonium sulfate concentration. All fractions were dialysed in water to remove the ammonium sulfate prior to analysis. Addition of the three fractions to the LOX inhibition assay showed that they all reduced the rate of reaction with the 80% fraction having the most significant effect (Figure 27). Further analysis of the fractions using the competitive ANS lipid-binding assay has shown that the 80% fraction also had the highest lipid binding capacity. These results could suggest that LOX was being inhibited by the presence of LBPs however, further experiments were required to prove that theory.

A particular concern with the LOX inhibition experimental protocol was that the linoleic acid was being completely consumed by the LOX during the course of the experiment. Therefore the rate of reaction was reduced, not because of an inhibitory effect brought about by the presence of LBPs but simply because there was no further linoleic acid for the LOX to act on. Therefore the LOX inhibition assay was performed with the protein extracts from malt 913P and 917P using double the concentration of linoleic acid. The presence of double the concentration of linoleic acid made no significant difference, with the rate of reaction decreasing in exactly the same way as was observed for the initial experiments (Figure 27).

Further experiments were conducted to identify whether LBPs are involved in the inhibition of LOX or to ascertain if another mechanism was responsible. Hydroperoxide lyase is an enzyme present in malt that converts hydroperoxides into aldehydes. Therefore the action of this enzyme would convert the reaction products of LOX into aldehydes preventing or reducing the measurement of the hydroperoxides by spectrophotometer. The protein extracts were all boiled for 30 min, as part of the extraction procedure, potentially deactivating any enzymes present, however, it was necessary to determine that there was no active hydroperoxide lyase in the protein extract. Hydroperoxides were formed in a cuvette by the action of LOX, protein extracts were added to the cuvette and any change in absorbance measured using a spectrophotometer. The reaction was measured for a period of 10 min and during that time there was no change in absorbance compared to the control (data not shown). This result would suggest that there are no active hydroperoxide lyases present in the protein extracts that have been prepared from barley and malt. However, there is still a question as to whether cereal LBPs are involved in reducing the activity of LOX. There is no doubt that a LBP can reduce the activity of LOX, BSA has demonstrated that, but further work is necessary to establish a similar property in cereal LBPs.

3.6.4 Trans-2-nonenal Formation in Artificially Aged Beer

The influence of LBPs, to influence the flavour stability of beer, was assessed by measuring their effect on the formation of trans-2-nonenal in stored beer. A pilot brewed lager style beer was spiked with 2 mg/mL of BSA or protein extracts from barley and malt. The beer was artificially aged as described in the materials and methods and the trans-2-nonenal concentration measured by GC-MS. The control beer after ageing contained 0.45 µg/mL trans-2-nonenal, the beers spiked with protein extracts from barley and malt contained on average over 50% more trans-2-nonenal than the control (Figure 28). However, the beer spiked with BSA contained 40% less trans-2-nonenal. The

increase in trans-2-nonenal when beer was spiked with the protein extracts from barley and malt could be due to the presence of fatty acids in the extracts. Therefore when they were added into the beer the levels of oxidisable fatty acids increased, resulting in an increased formation of trans-2-nonenal when compared to the control. It would be interesting to repeat this experiment with a defatted LBP from either barley or malt. However, the BSA being a pure protein and therefore fatty acid free has had a positive effect on the trans-2-nonenanl level reducing it significantly when compared to the control. The exact mechanism by which the BSA affects the concentration of trans-2-nonenal is not known, it may be that bound fatty acid is not as oxidisable as free fatty acid, further work is required to understand this fully. However, there is the possibility that lipid-binding proteins can have a positive effect on beer flavour stability by reducing the oxidation of fatty acids in stored beer.

3.5.6.5 Conclusion

LBPs have been shown to have both a positive and negative influence on the flavour profile of beer. In studies using the BRi sensory panel, increasing concentrations of BSA, a model LBP, have been shown to effect negatively the aroma profile of beer, with an increase in cheesy, rancid, solvent and musty notes. This may be due to the fact that the BSA was binding the positive aroma notes allowing the negative aroma notes, which are usually masked, to be perceived by the sensory panel.

However, with respect to trans-2-nonenal LBPs may have an important role to play. BSA and protein extracts from barley and malt have been shown to inhibit LOX activity. However, further work is required to discover if the inhibitory effect due to the protein extracts from barley and malt is caused by protein or some other non-proteinaceous material present in the extract. This would require more thorough and exhaustive protein purification procedures to be employed. LBPs have also been shown to influence positively the formation of trans-2-nonenal in beer that was being stored. The presence of BSA reduced the concentration of trans-2-nonenal by approximately 40% when compared to the control after 1 week of artificial ageing. Unfortunately the cereal LBPs used in this study had not been purified extensively and so may have introduced fatty acids into the beer, resulting in an increase in the formation of trans-2-nonenal rather than the desired decrease.

It is necessary to repeat some of the experiments described above with purified cereal LBPs if a full understanding of their effect on the flavour profile and stability of beer is to be achieved.

3.7 Acknowledgements
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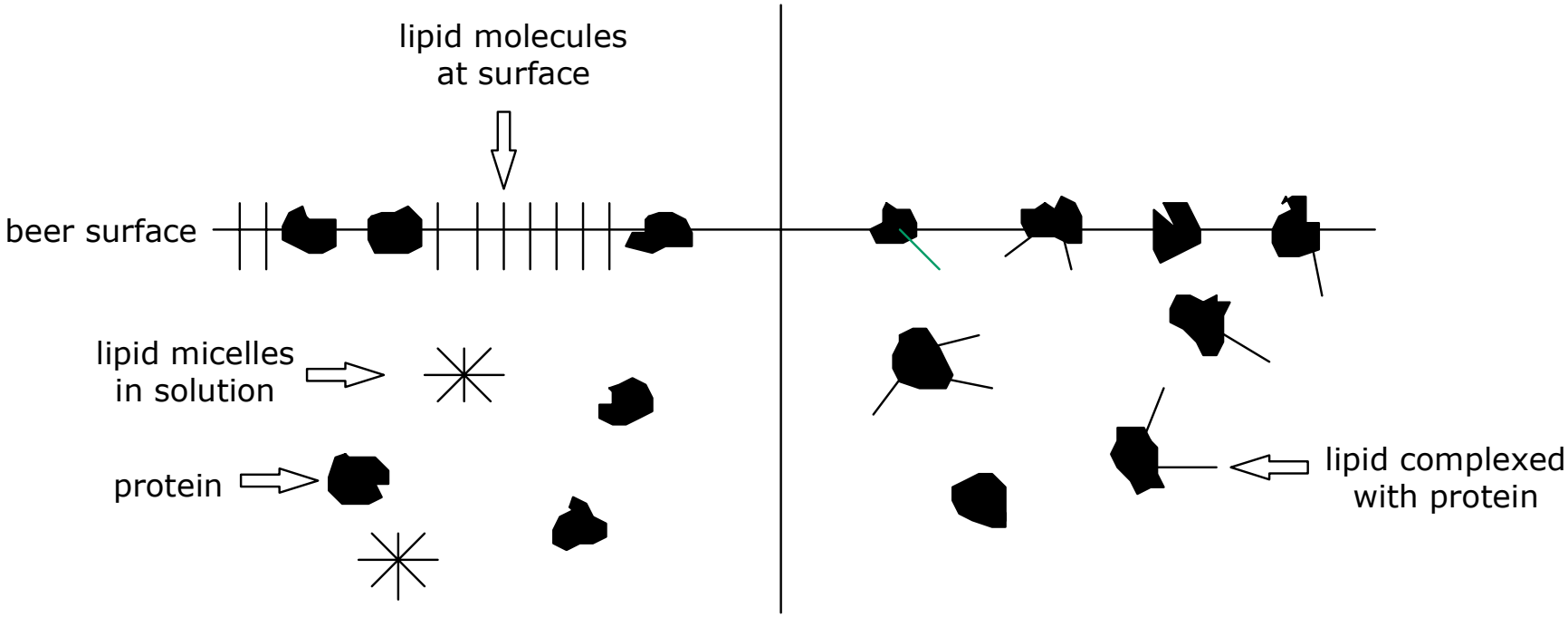
Figure 1. Influence of Lipids on Beer Foam



Initial State
lipid exists independently and enters bubble wall, causing collapse of beer foam

Final State
lipid becomes associated with other substances, mainly protein, in beer and does not destroy foam.

Figure 2. Probe Fluorescence Method

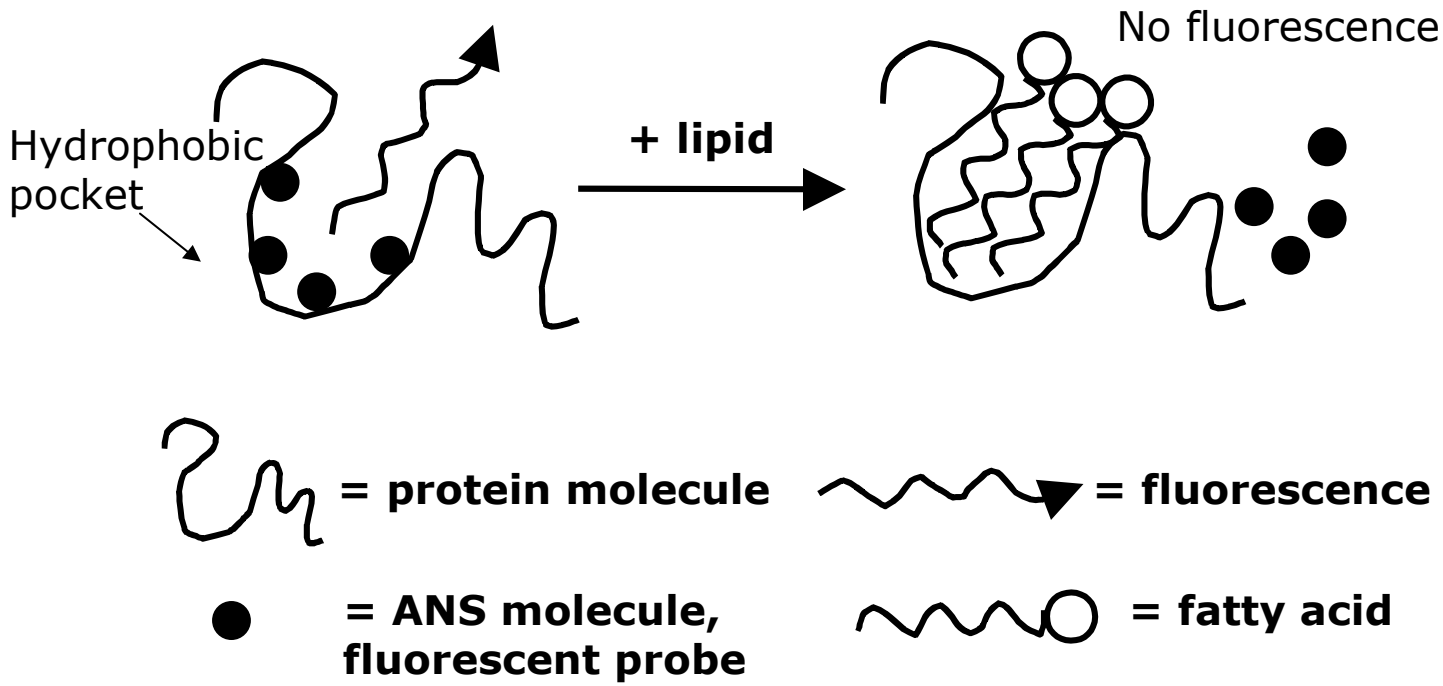


Figure 3. Effect of fatty acid addition on ANS fluorescence of BSA

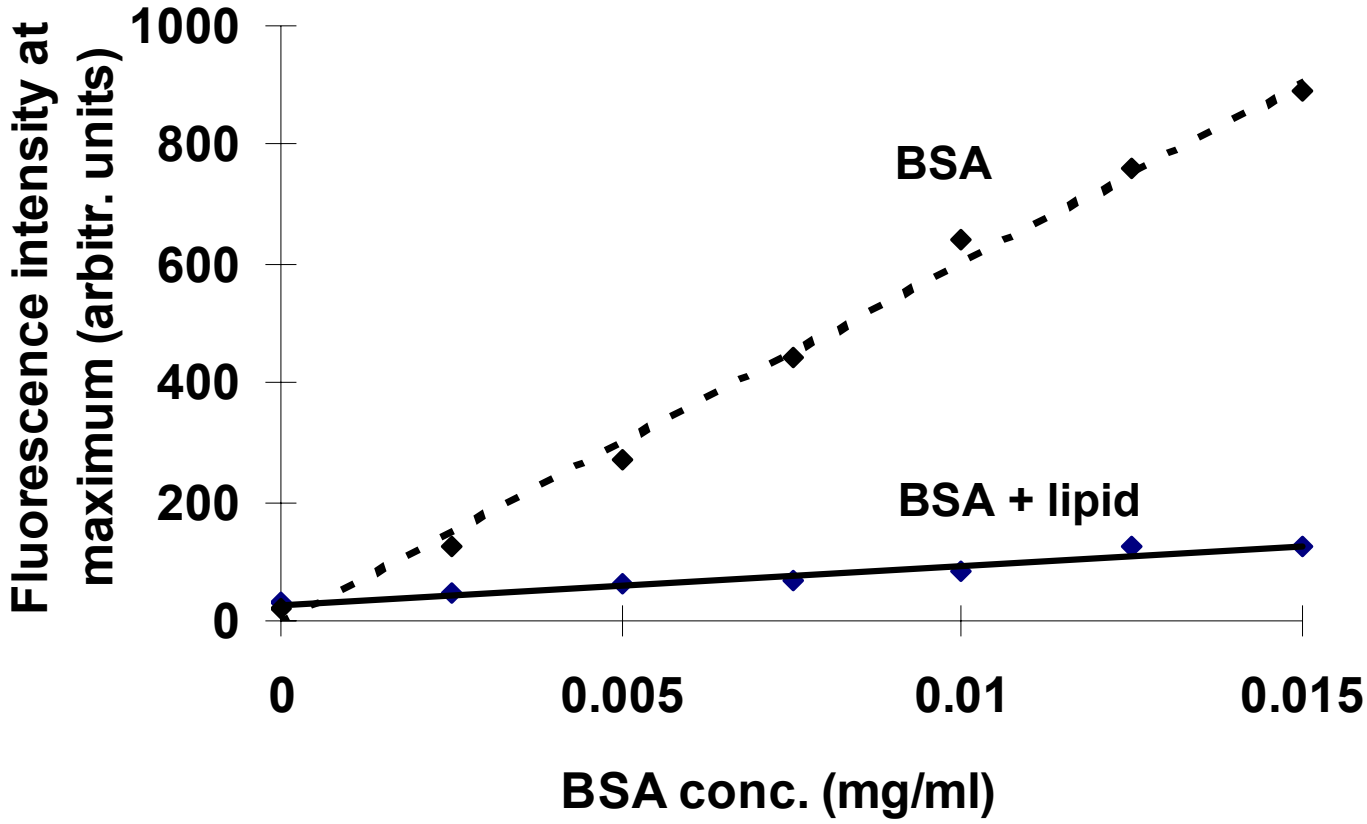


Figure 4. Effect of fatty acid addition on ANS fluorescence of barley soluble protein extract

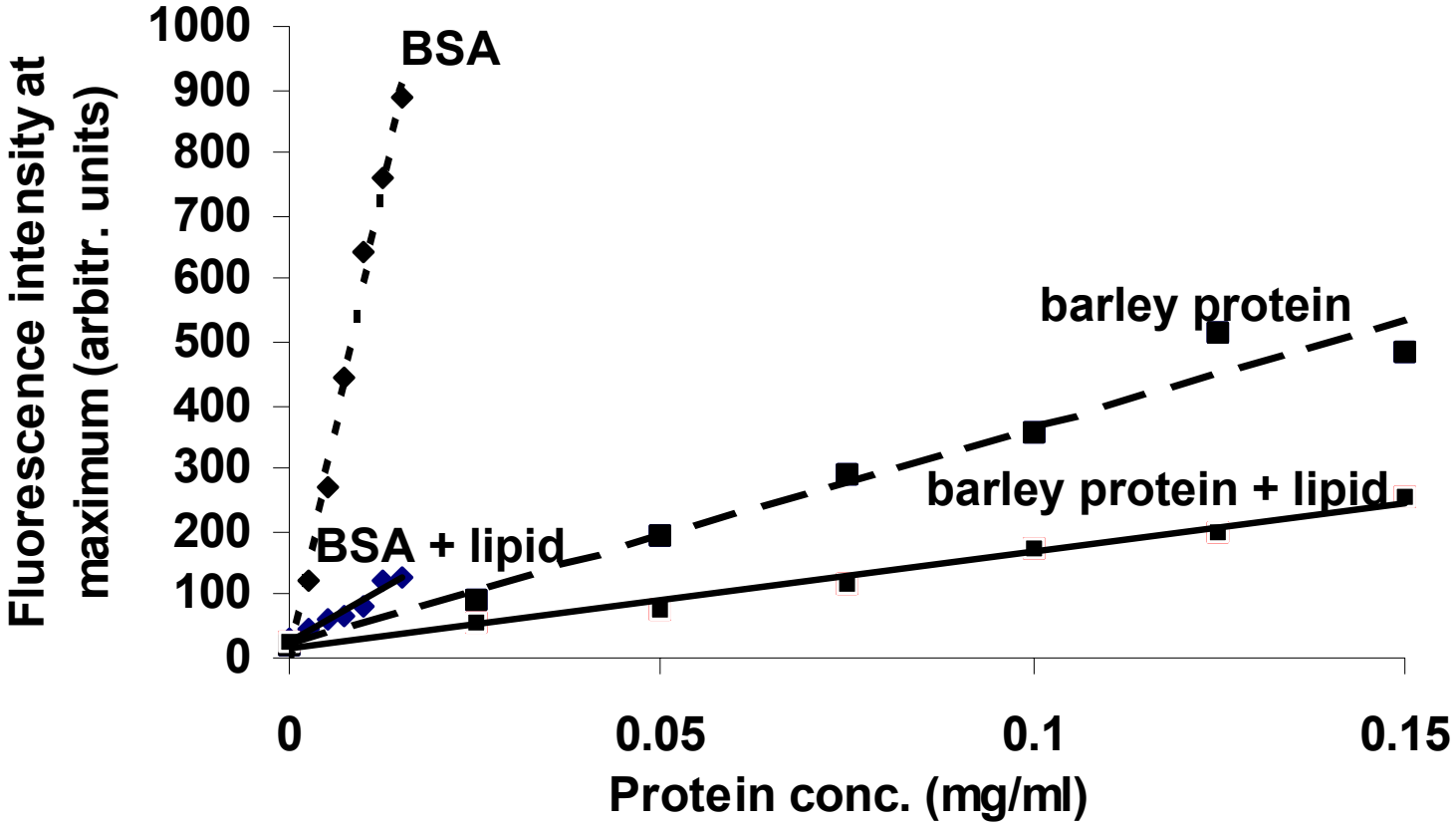
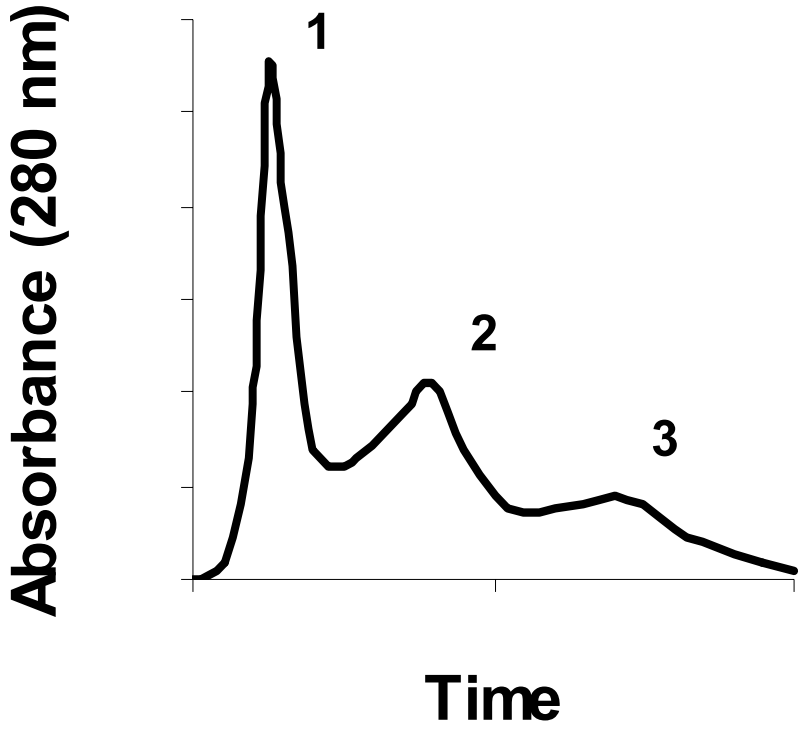


Figure 5. FPLC of barley soluble extract



- **Peak 1**: High MW molecules (proteins)
- **Peak 2**: Medium MW molecules (polypeptides)
- **Peak 3**: Low MW molecules (amino acids)
- Peak 2 + 3 do not show FA-binding in ANS test

Figure 6. Absolute lipid-binding capacities of feed and malting barleys

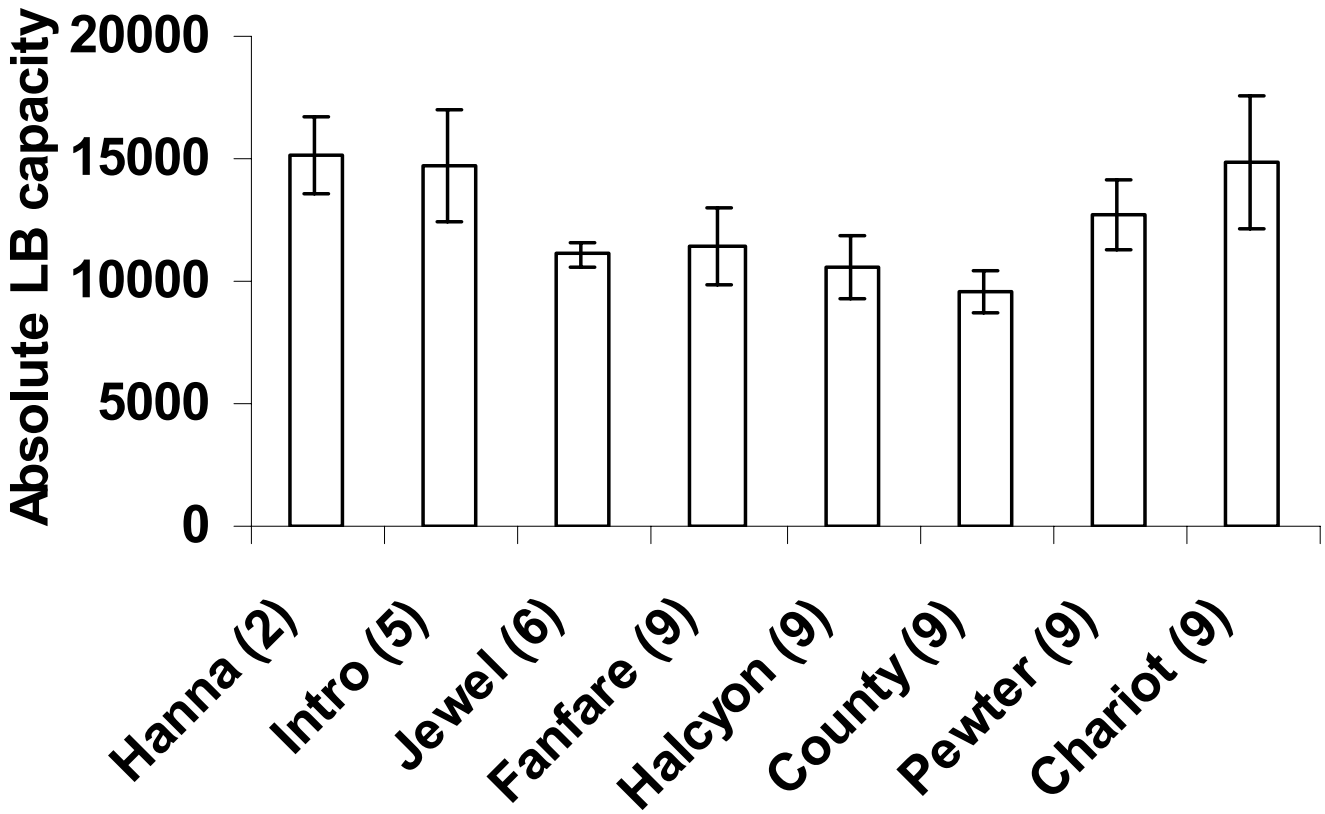


Figure 7. Comparison of barley and malt absolute lipid-binding capacities

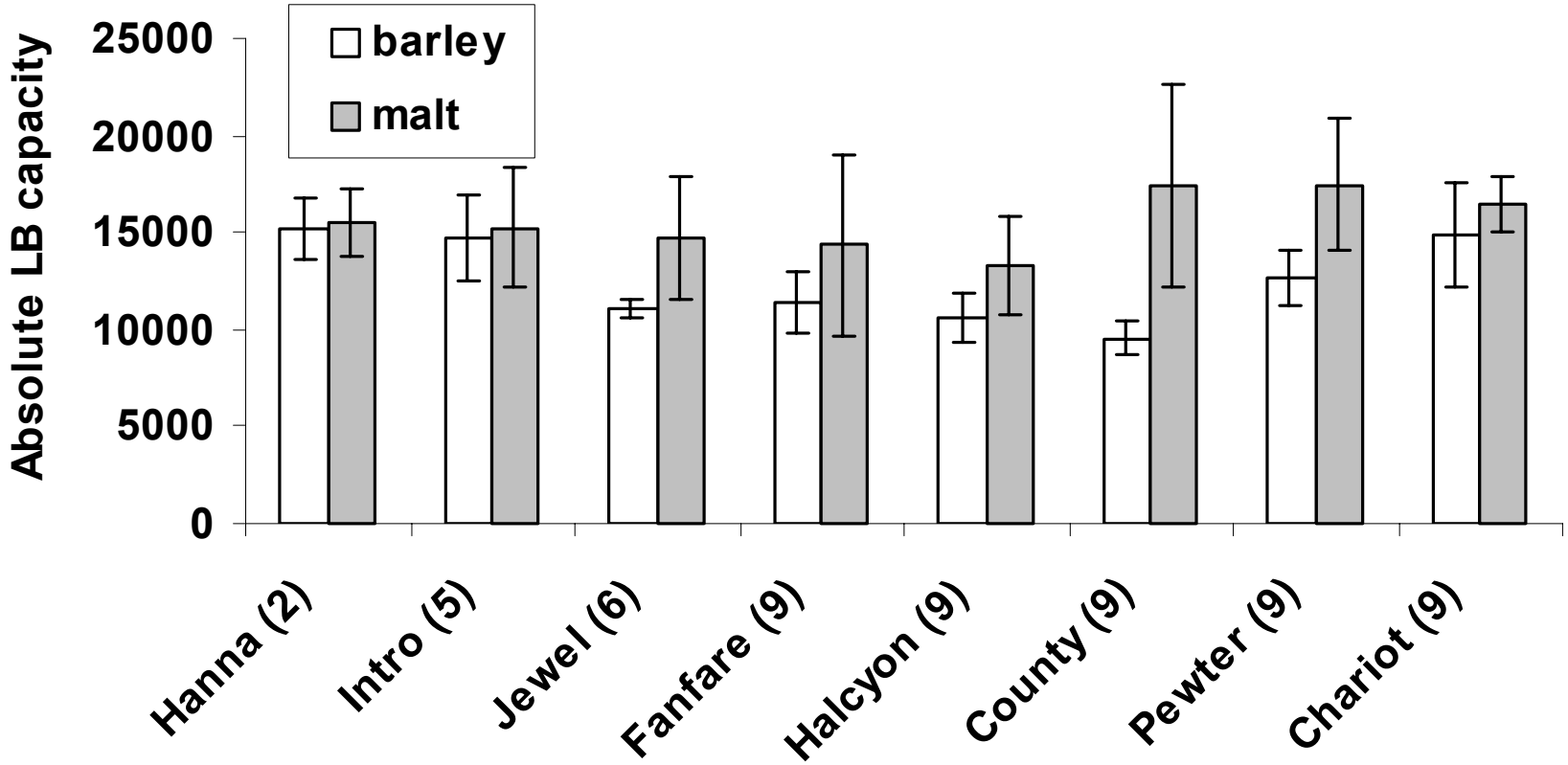


Figure 8. Absolute lipid-binding capacities of Chariot barley with different TN

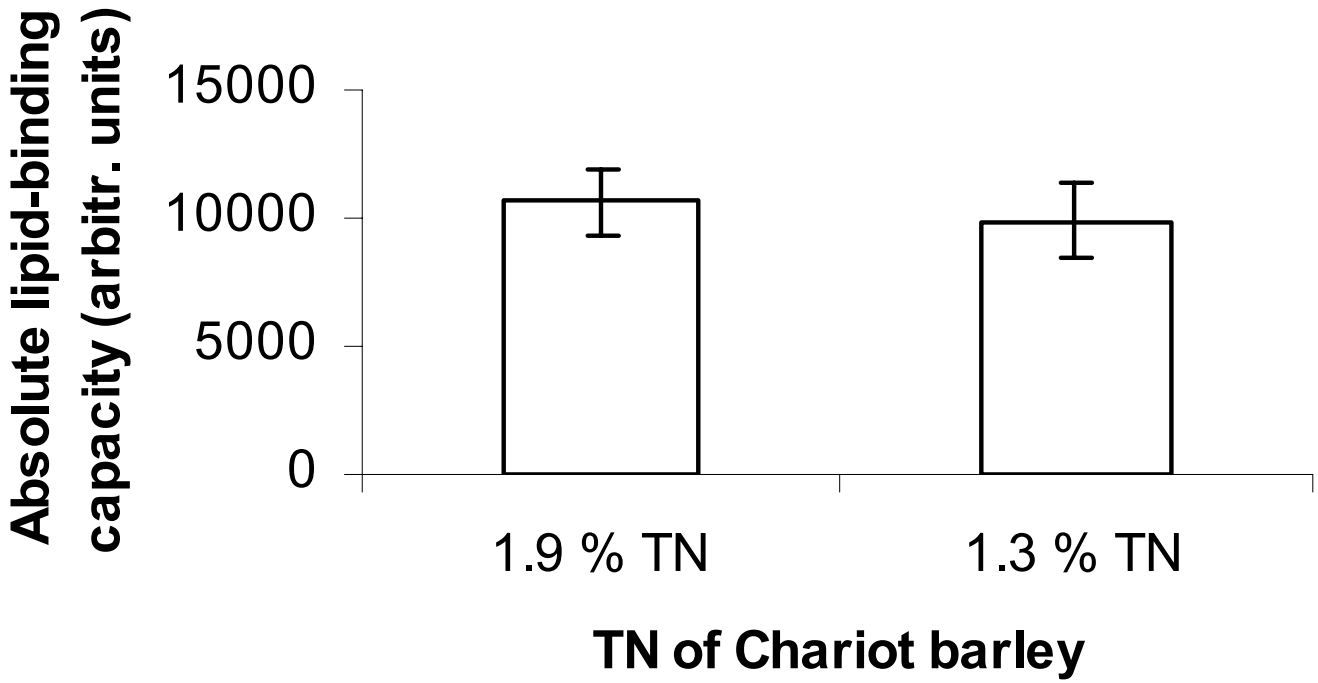


Figure 9. Malting time course

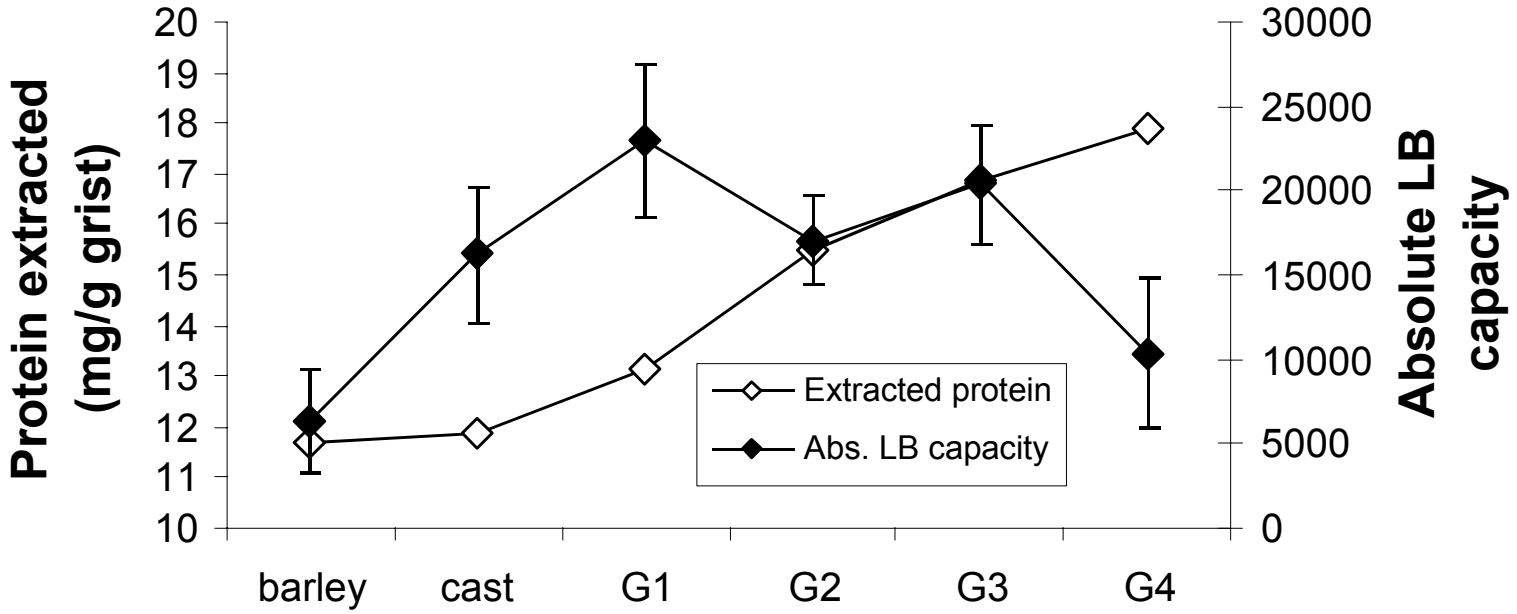


Figure 10. Absolute LB capacity of Hanna (grade 2)

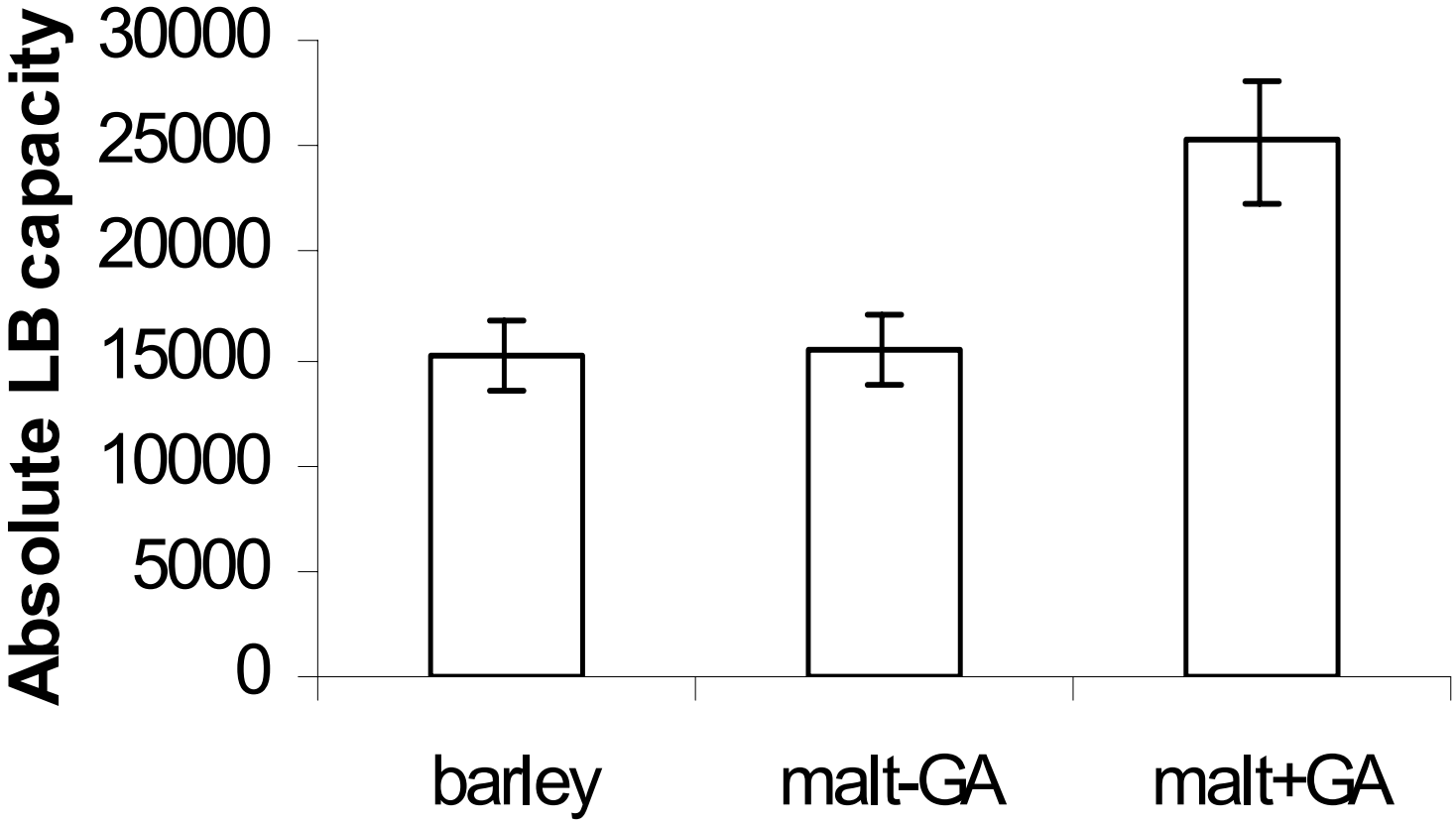


Figure 11. Absolute LB capacity of Jewel (grade 6)

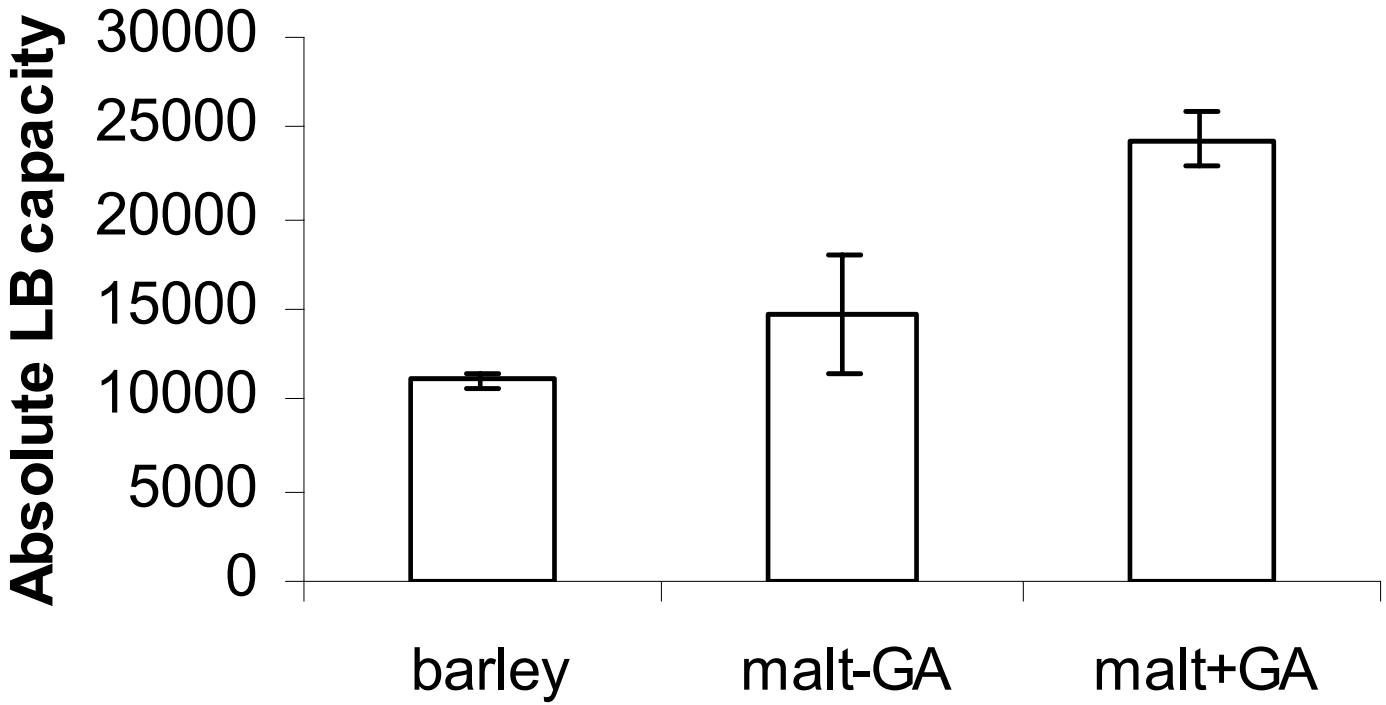


Figure 12. Absolute LB capacity Fanfare (grade 9)

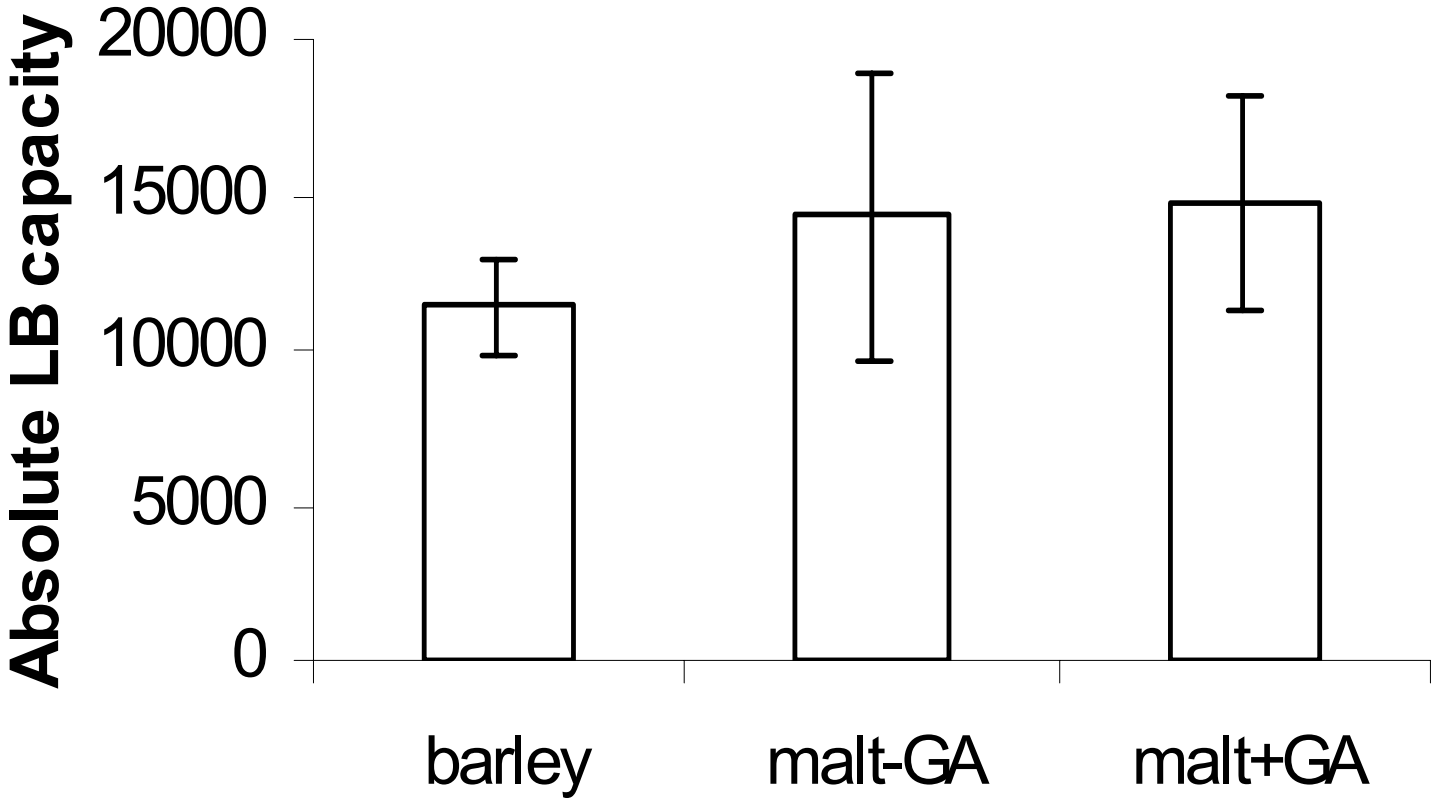


Figure 13. Effect of kilning at different temperatures on LB capacity

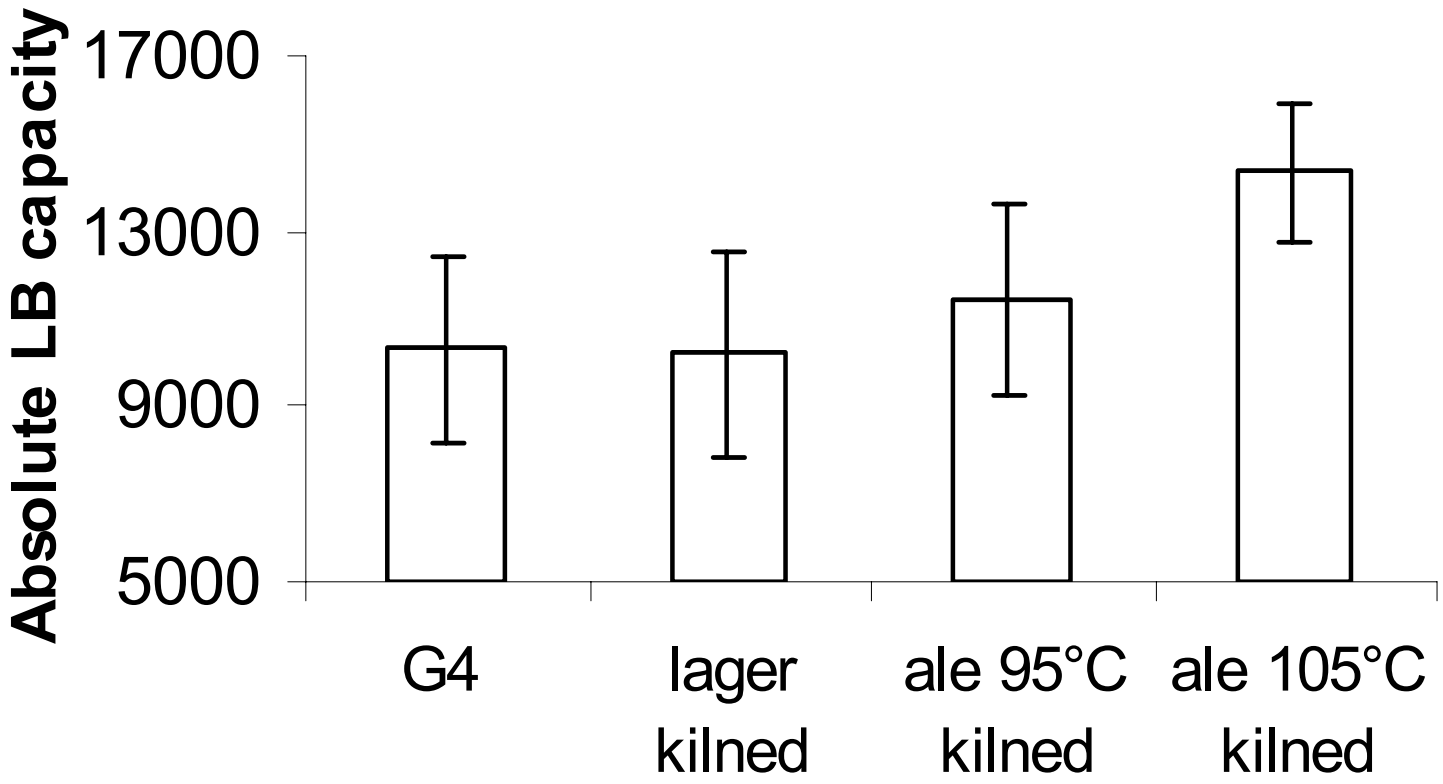


Figure 14. Levels of Fungi v LBP in different Barleys

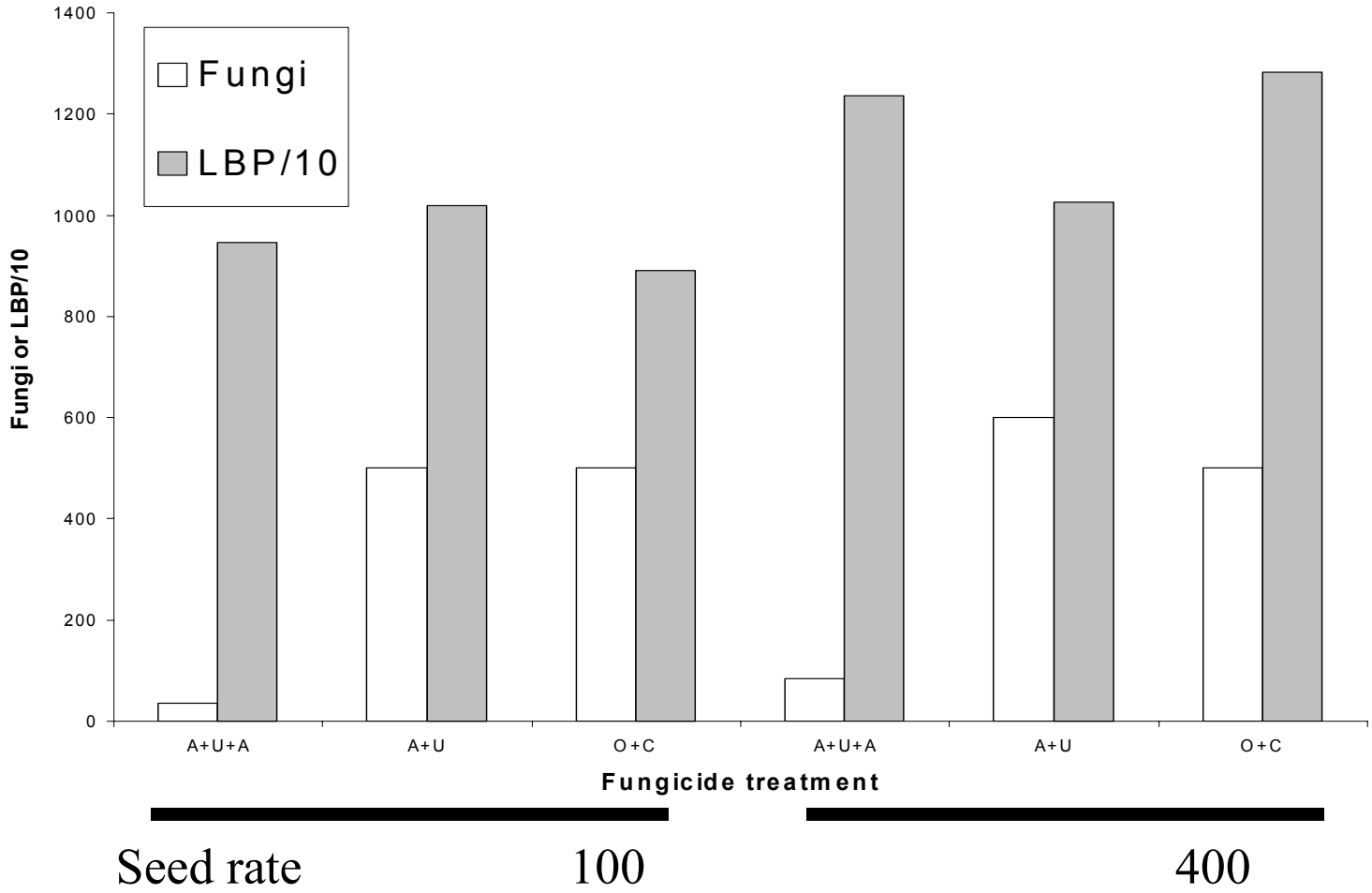


Figure 15. Levels of Yeast v LBP in different Barleys

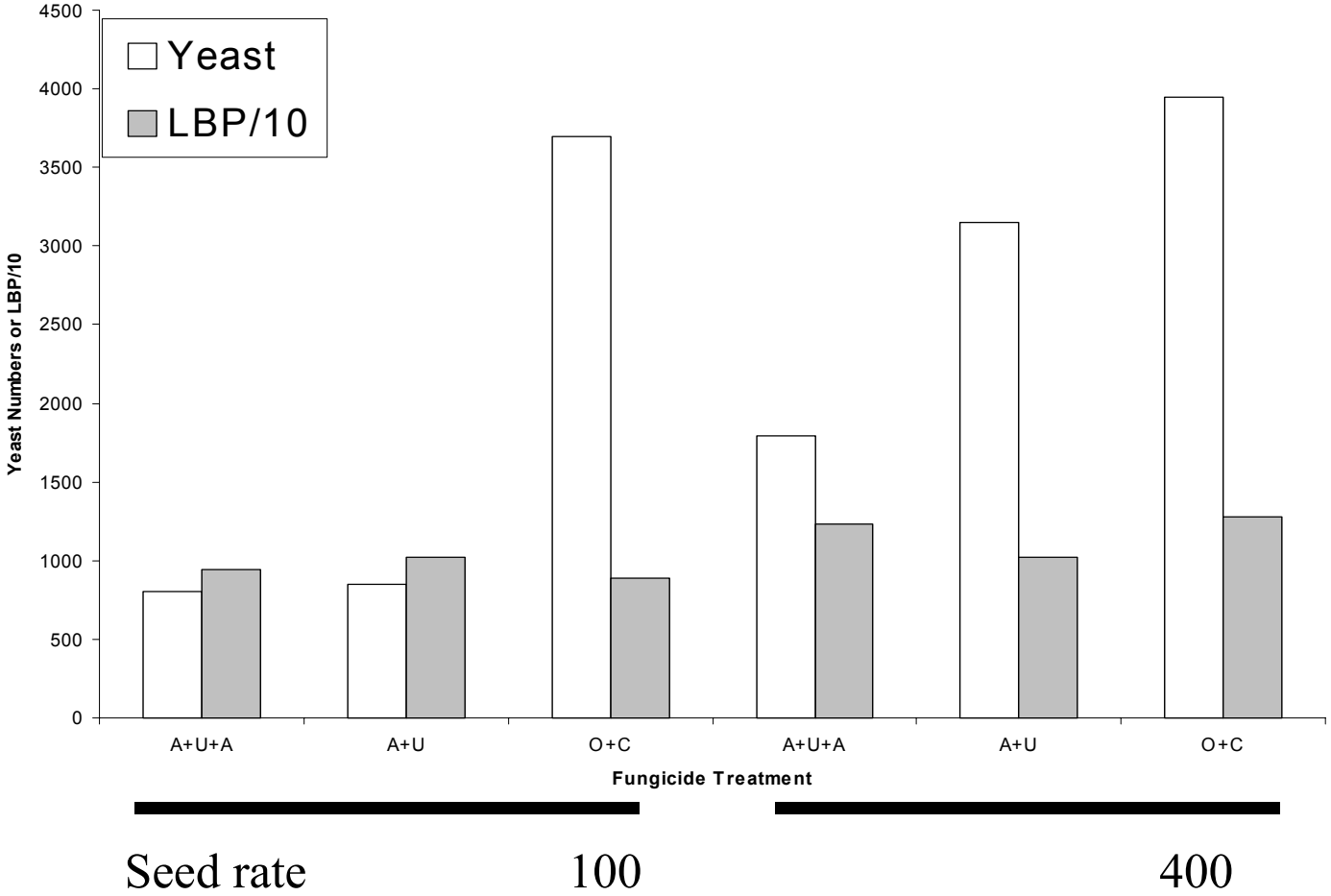


Figure 16. Absolute LB capacity of Optic barley at different seed rates

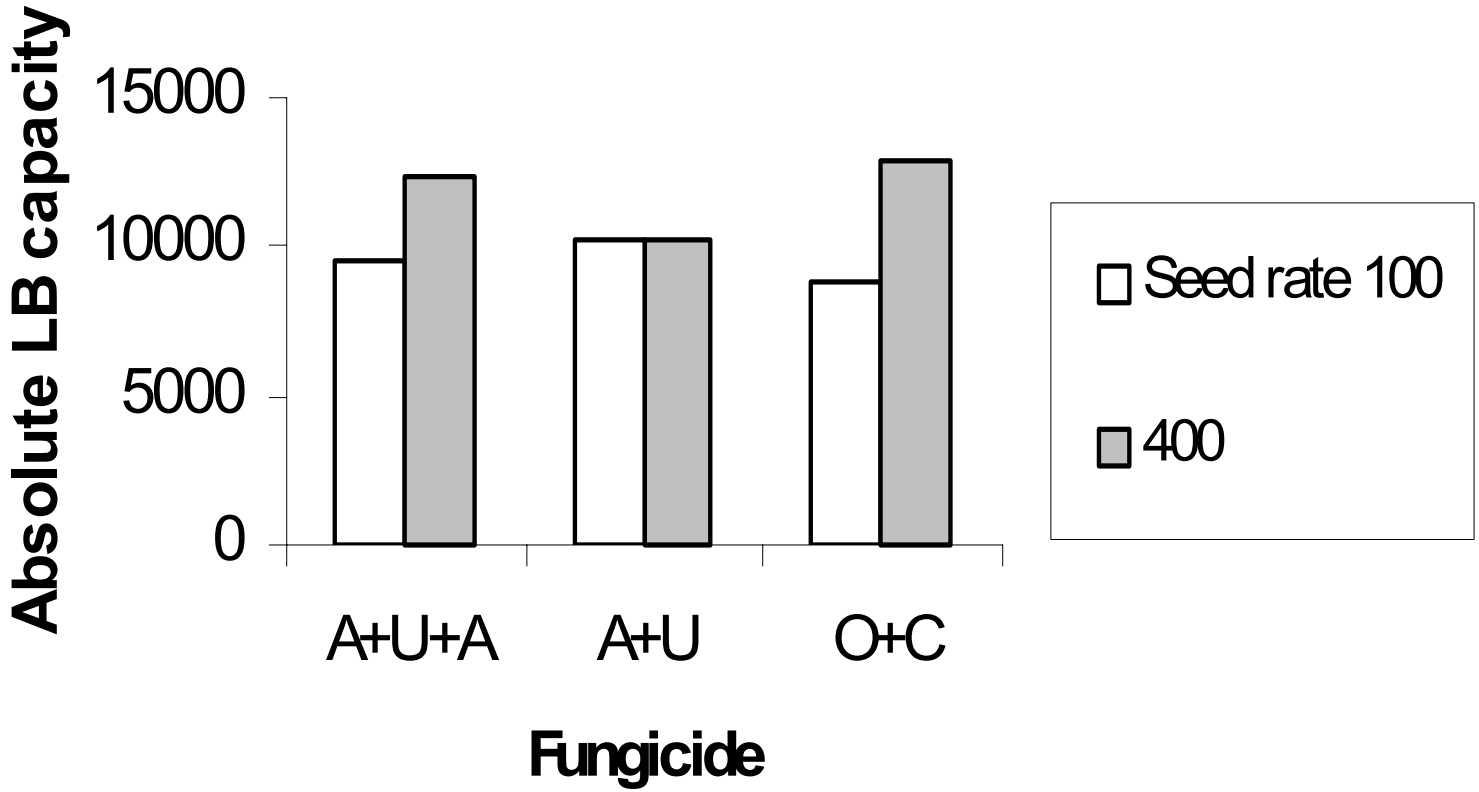


Figure 17. Effect of fungal growth on LB capacity

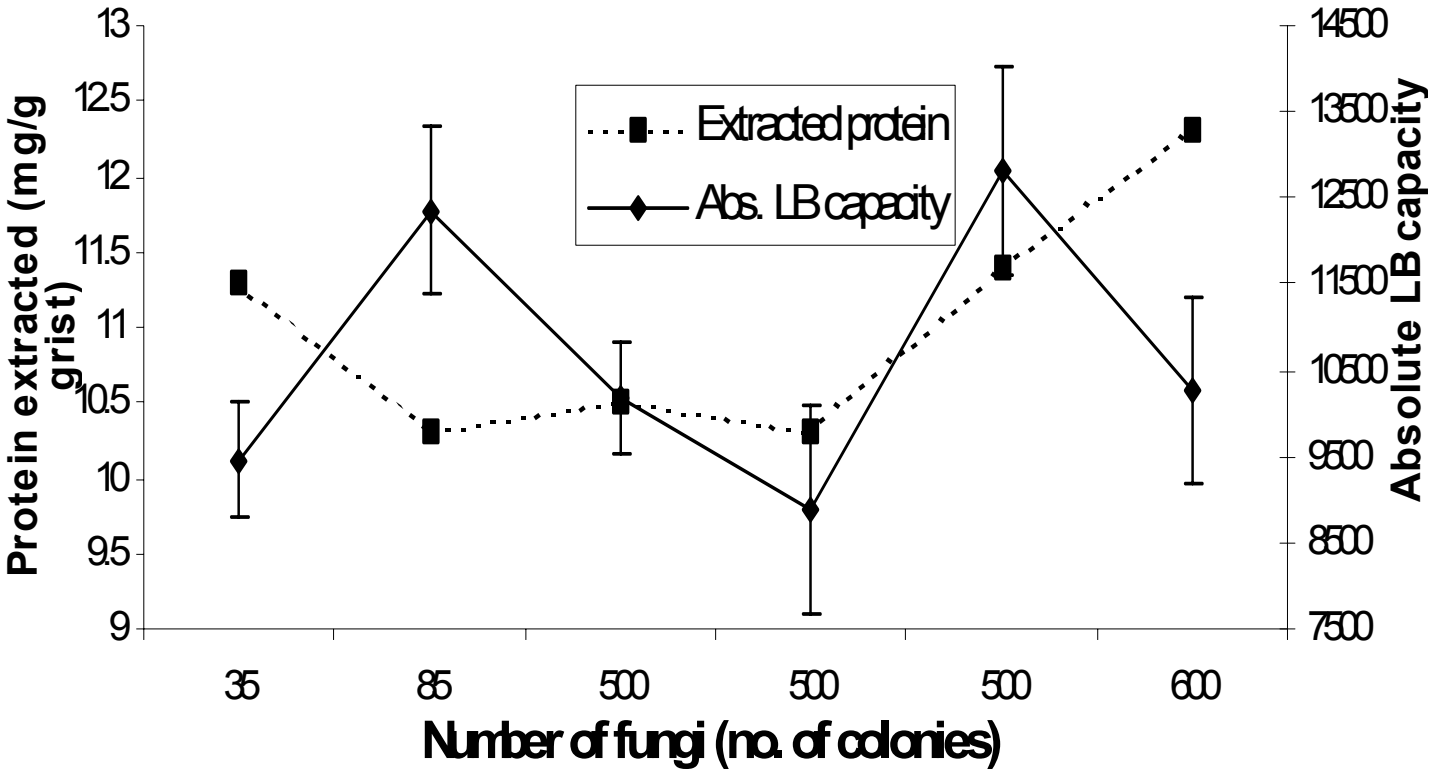


Figure 18. Time Course of Brewing Process

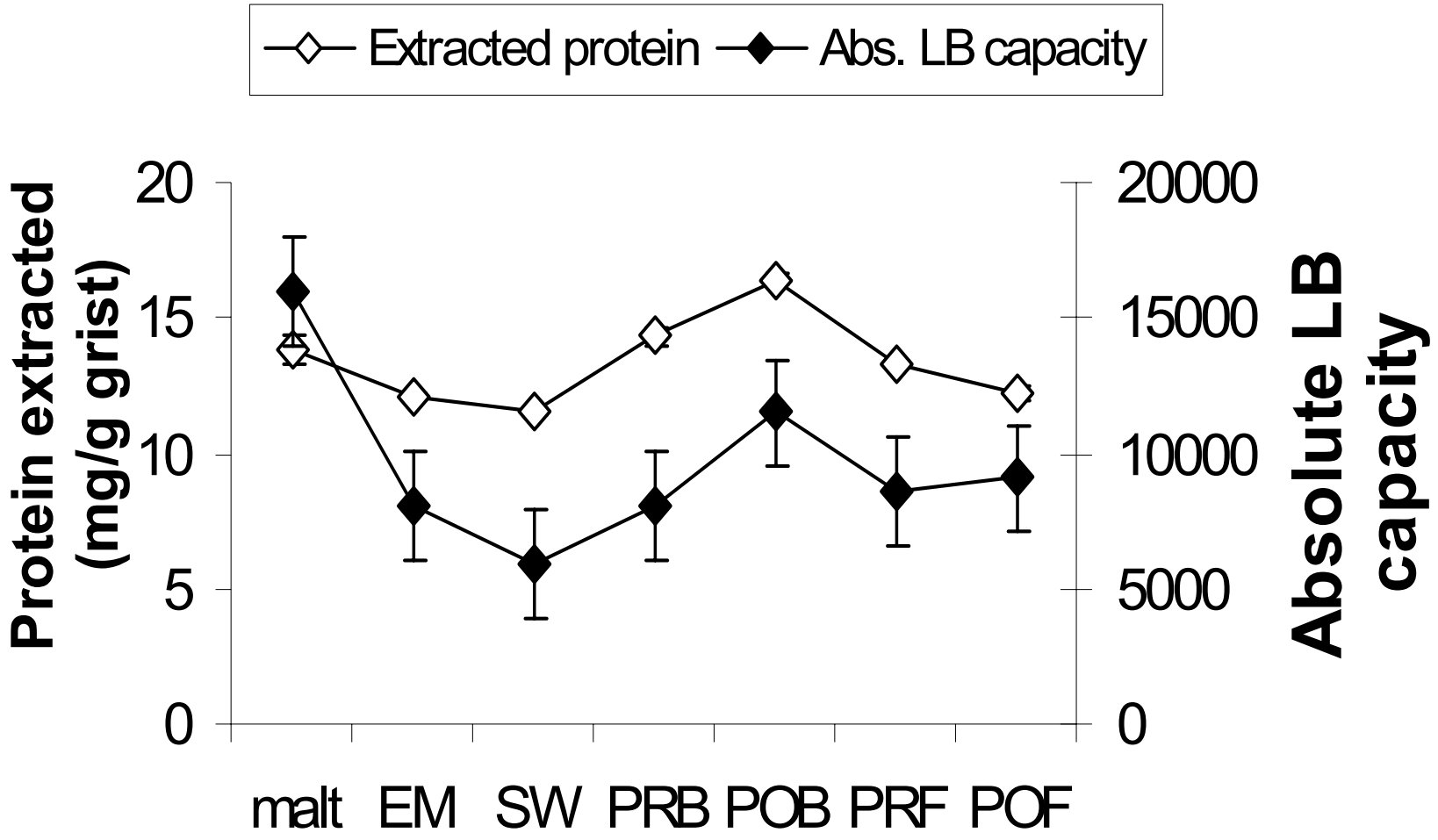


Figure 19. Effect of mash thickness on yield of LBP by ANS

Figure 20 LBP: Flavour binding I

AROMA PROFILES - BSA TRIAL

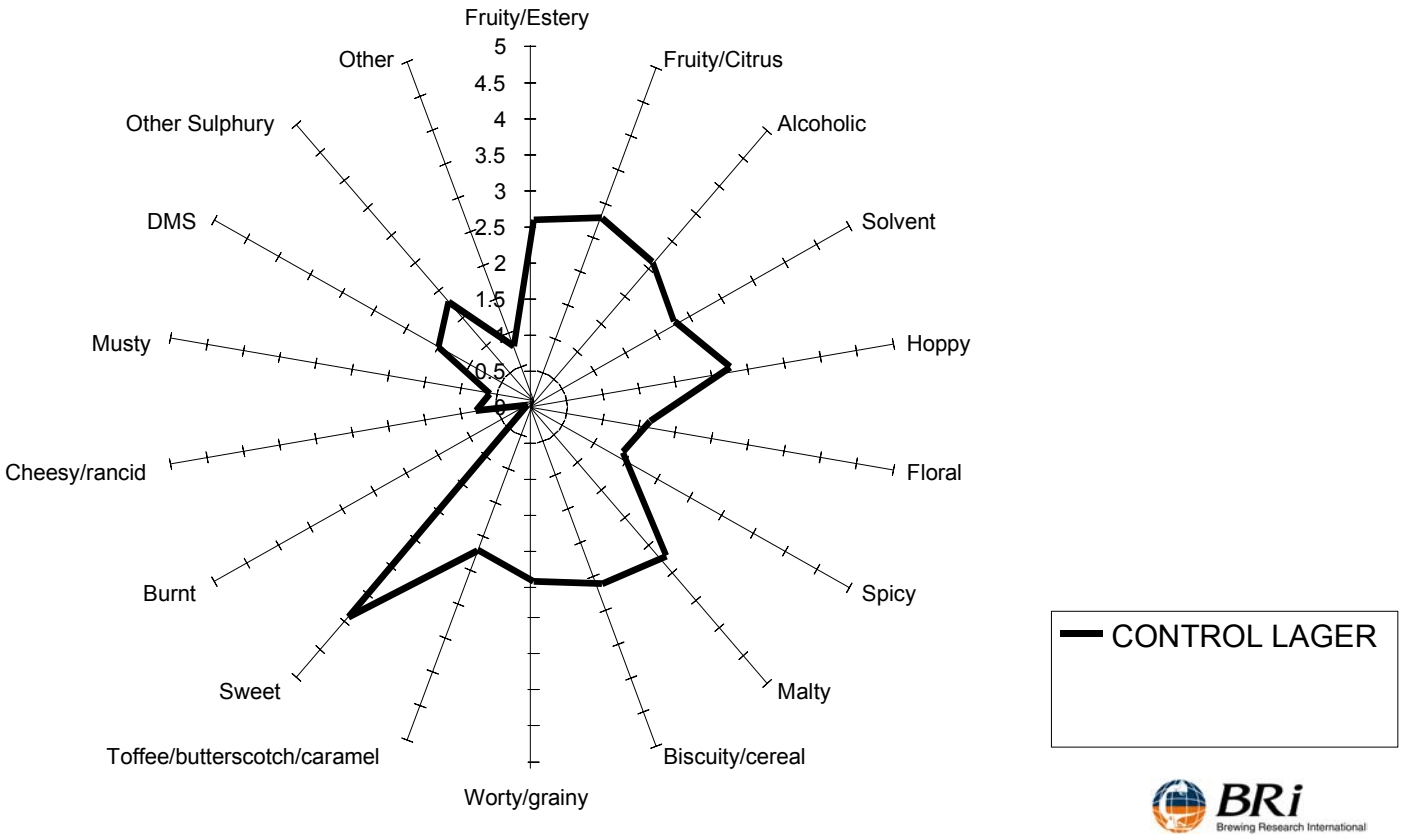


Figure 21 LBP: Flavour binding II

AROMA PROFILES - BSA TRIAL

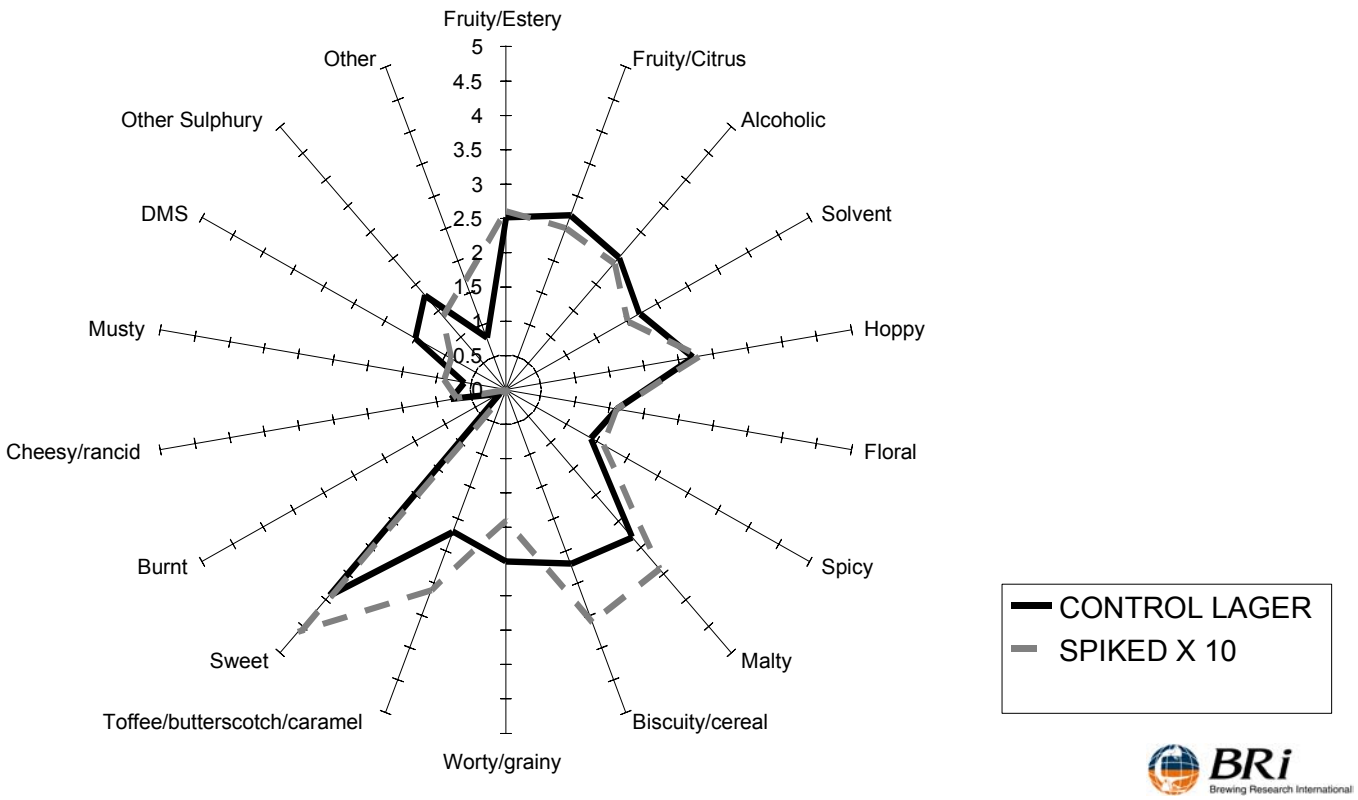


Figure 22 LBP: Flavour binding III

AROMA PROFILES - BSA TRIAL

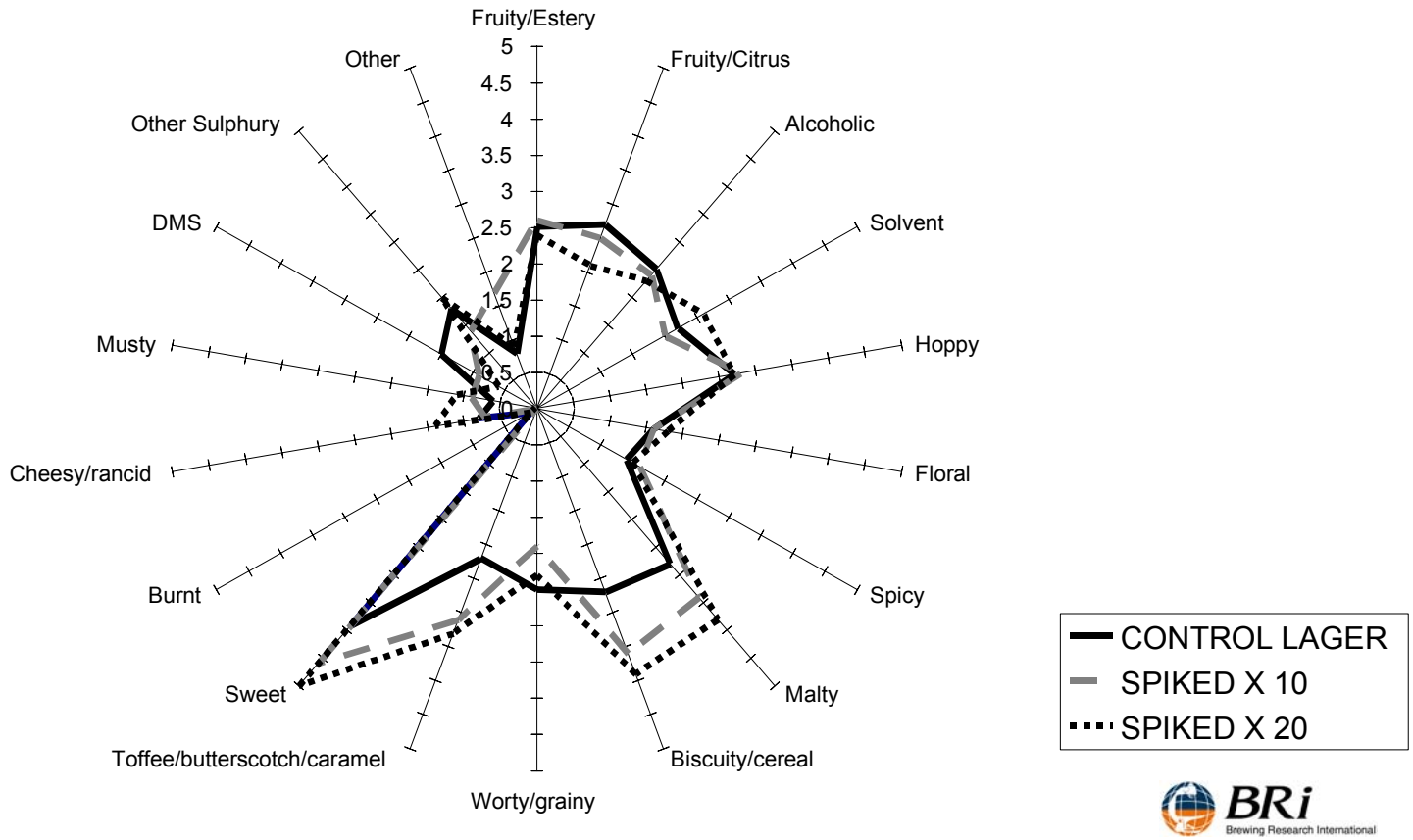


Figure 23. Percentage change of aroma compounds measured in control beer and beer spiked with BSA.

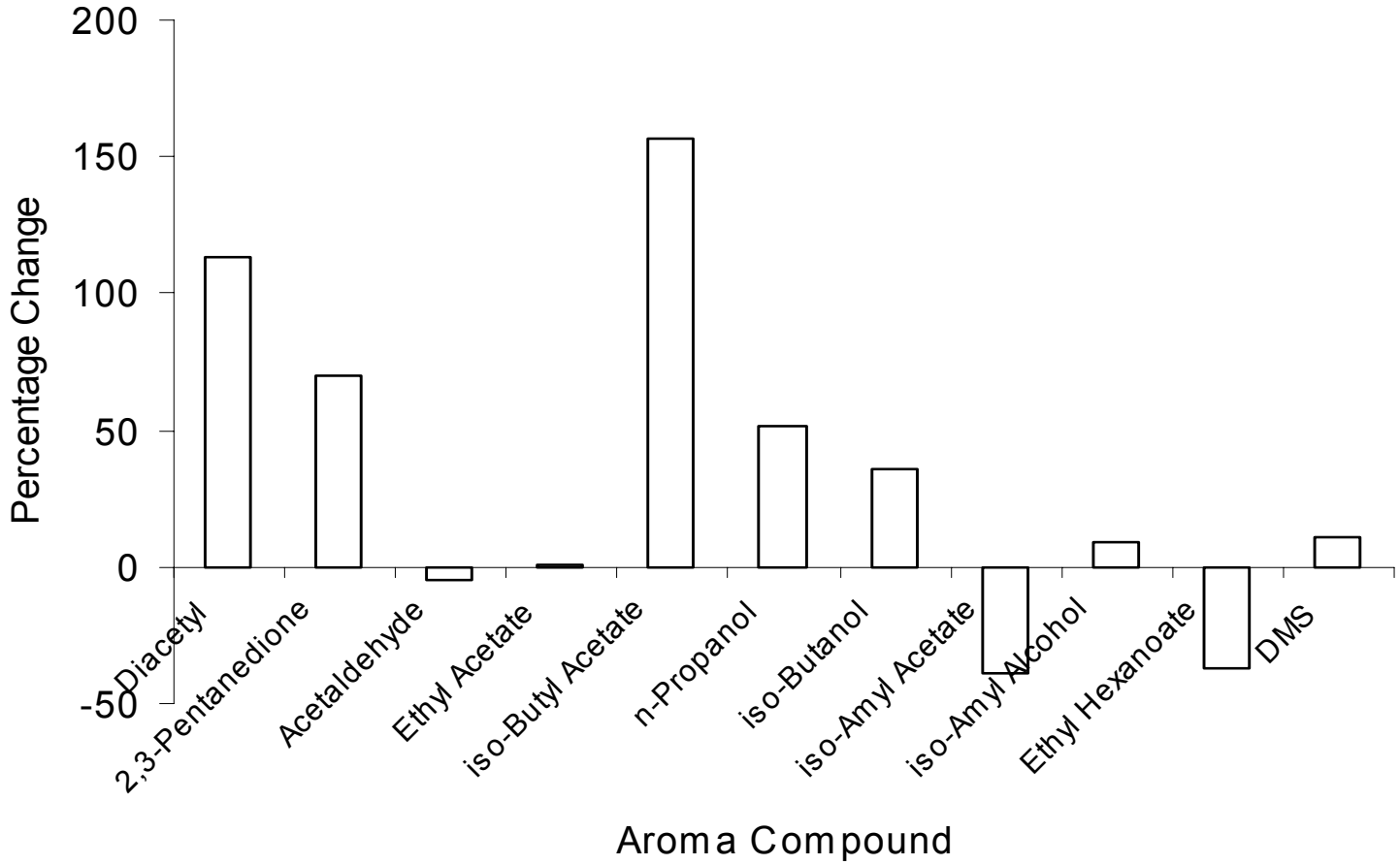


Figure 24. Percentage change of aroma compounds measured in control beer and beer spiked with wheat protein extract.

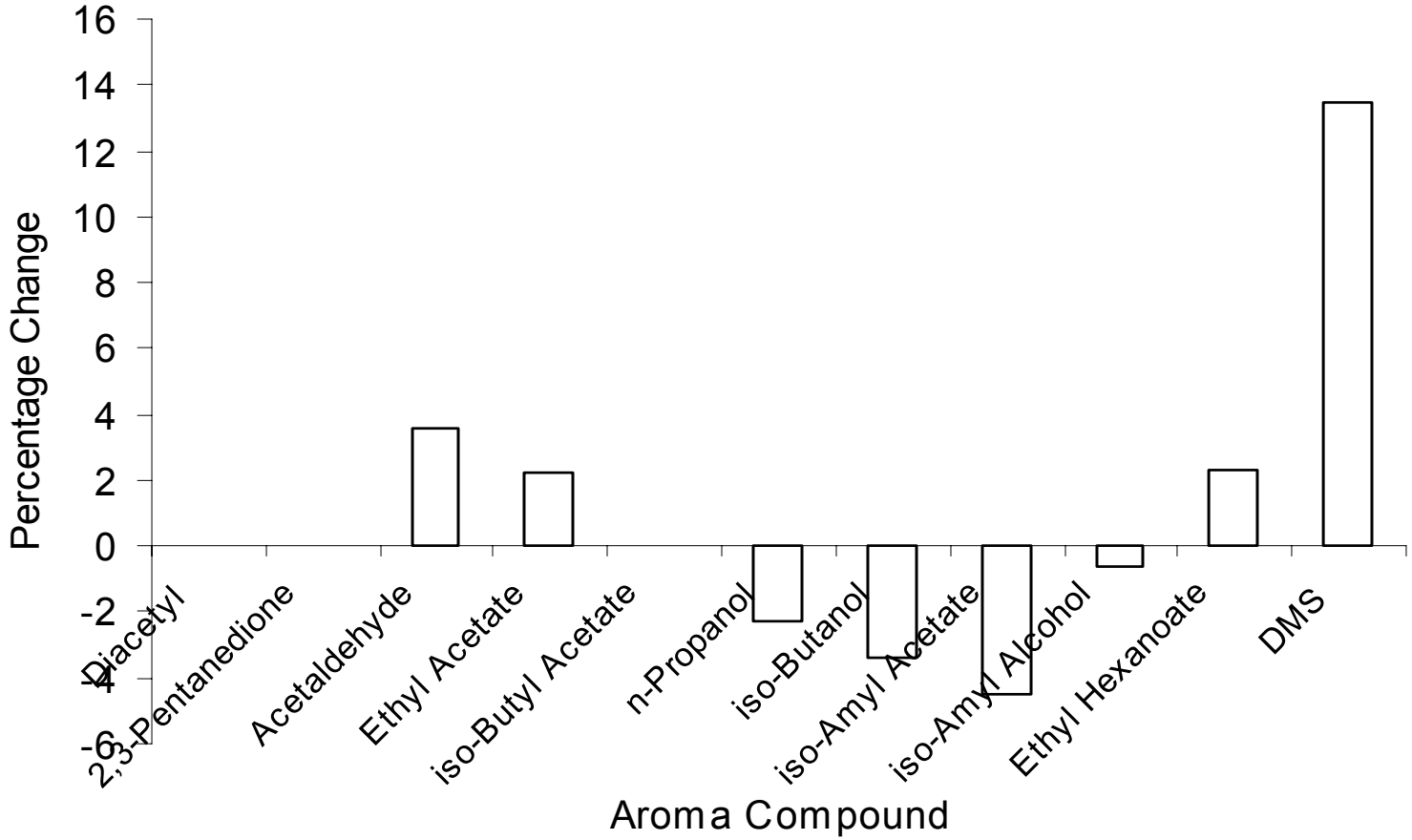


Figure 25. The effect of protein extracts on LOX activity.

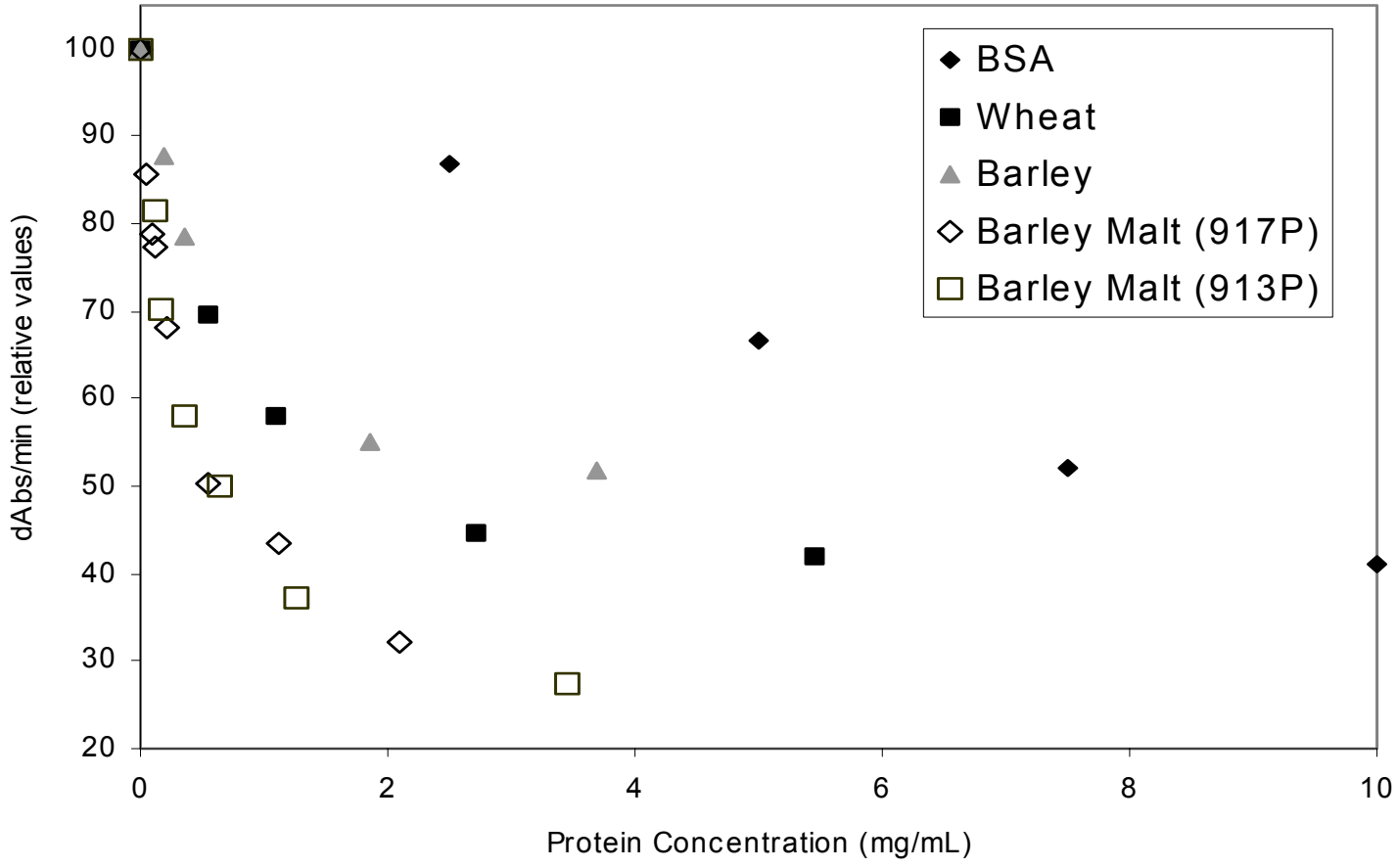


Figure 26. The effect of protein extracts on LOX activity.

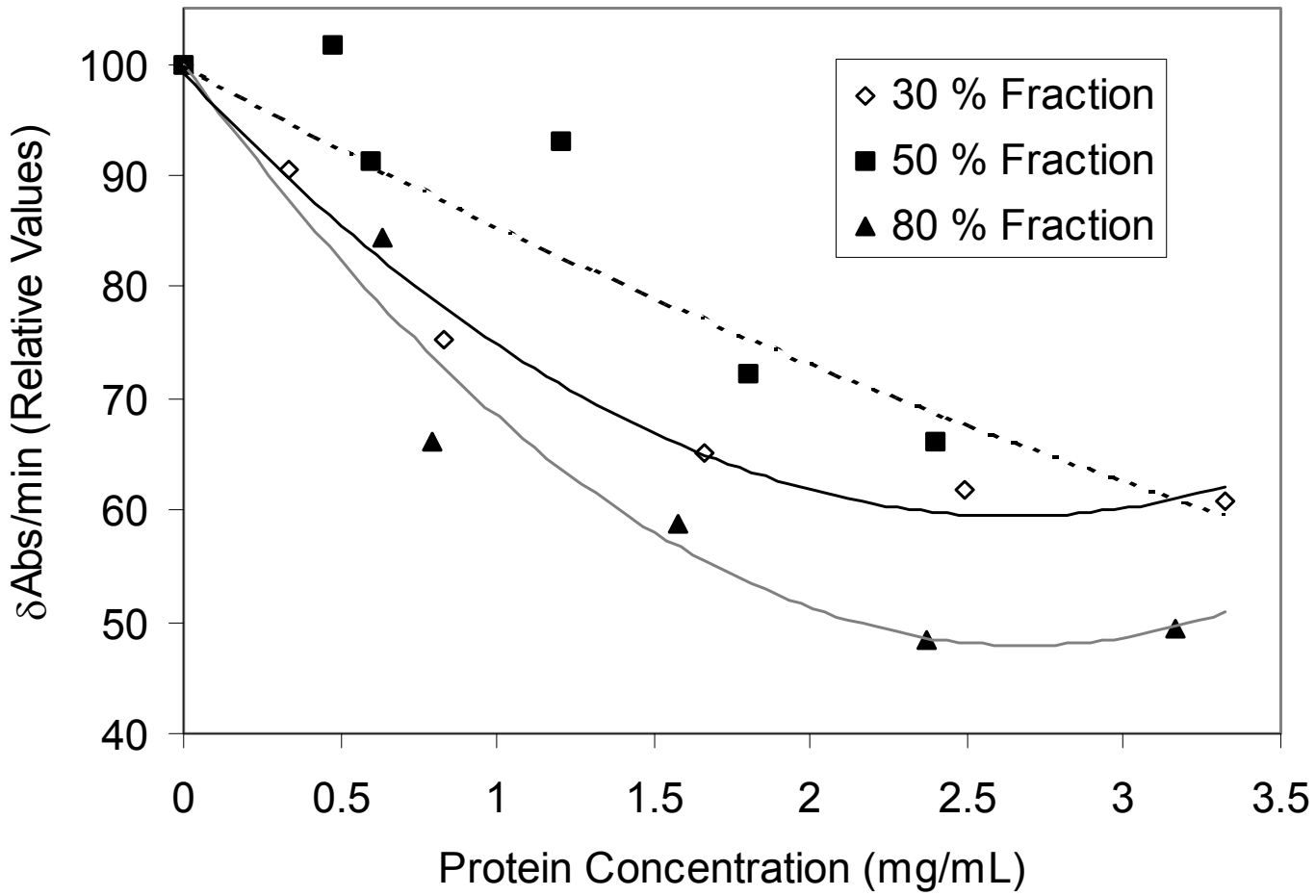


Figure 27. The effect of increasing linoleic acid (LA) concentration on the LOX inhibition assay.

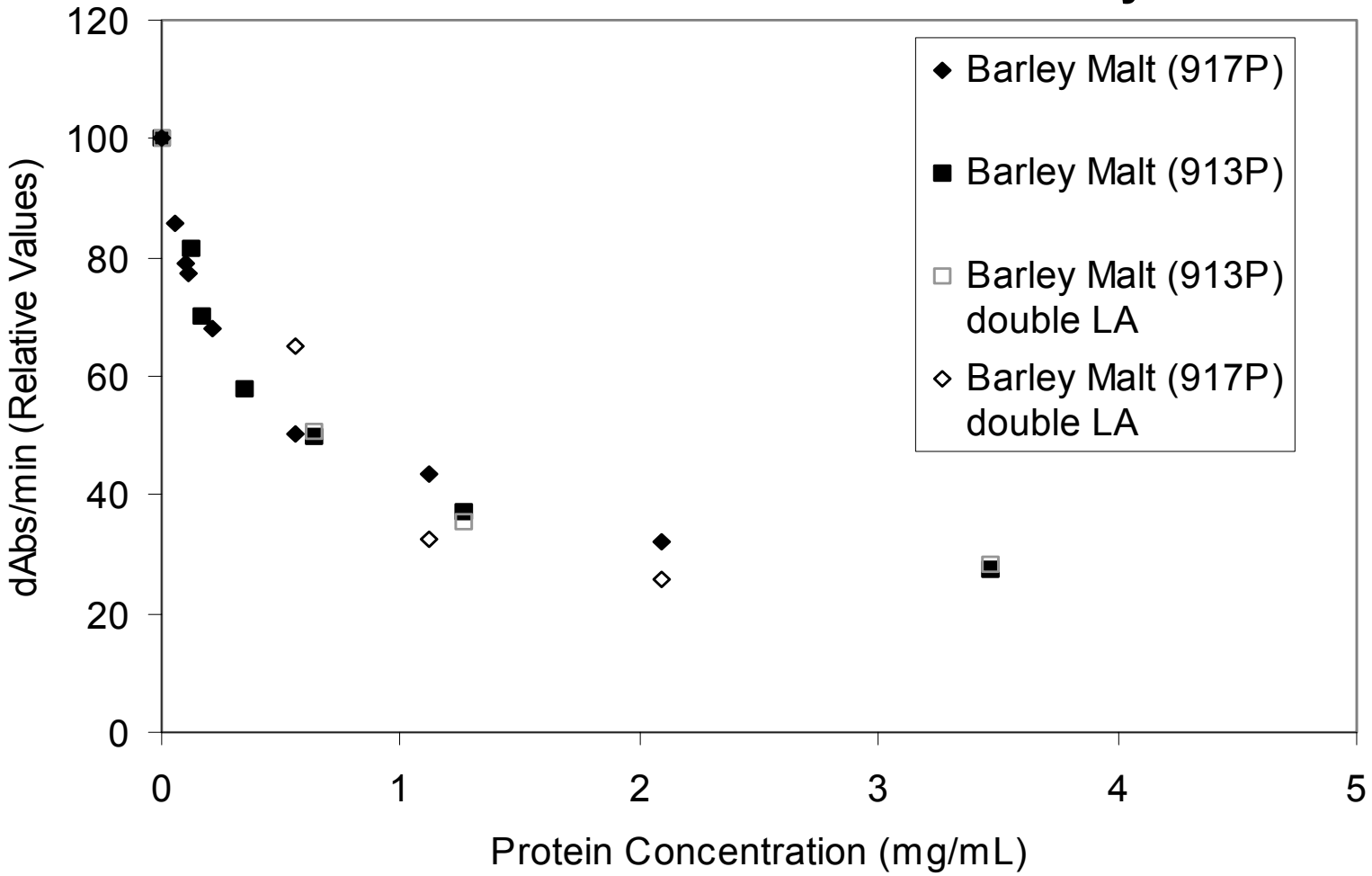


Figure 28. The effect of LBPs in the formation of trans-2-nonenal in artificially aged beer.