Effect of Manganese and some FAN supplements on fermentations

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In order to consider the role of manganese in yeast nutrition a full understanding of the effect of zinc is essential. For practical brewing the challenges of super high gravity and refined sugar addition require a further look at metal ion and nitrogen supplementation of wort. Faster fermentation and diacetyl reduction are demanded at the same time. Yeast foods have become important again and optimum fermentation depends, as always, on the overall nutrient state of the wort. This work applies some of the learning of academic research work to the practical problems of fermentation Manganese between 2 and 4 µM (0.011-0.022 mg/l) is required and above 10 mM (550 mg/l) becomes inhibitory for yeast fermentation. Manganese plays a role in stimulating protein assimilation which becomes vital when free amino nitrogen (FAN) is deficient in wort. Above 7µM (0.038 mg/l) manganese is essential for zinc assimilation and zinc is vital to prevent fermentations from approaching attenuation slowly. (Jones & Greenfield 1984). Manganese comes mainly from the grain but brewing water from deep boreholes frequently has high levels. Manganese may occur in hop extracts as it is used to catalyze the isomerisation of pre isomerised kettle extract. Unmalted barley has a 40% higher level of manganese that malted barley (Table 1) because metals are lost in the roots and shoots discarded after malting. In full scale breweries manganese is reduced in the aeration and sand filtration elements of water treatment as well as in the brewhouse kettle trub. Walker, De Nicola, Starley, & Learmonth, (2006) showed that magnesium and zinc and had a role in membrane systems but drew attention to changes in concentration due to losses in trub. This observation explains variable results in fermentation trials due to uneven distribution of metals.

The interactions between nutrients in brewery fermentations remain very complex because individual observations are specific to the yeast and exact fermentation conditions. Reproduction of results using naturally occurring materials is very difficult. Cereal varieties, growing season, soil fertility, crop rotation and fertilizer application all combine to alter the metal ion content of cereals as demonstrated in table 1 in this study. De Nicola, Starley & Learmont (2009) and Rees & Stewart (1998) observed strain differences in the response of yeast ester formation to zinc preconditioning of yeast. This shows that metal response findings are not identical across every situation especially when metal induced interactions are taken into account.

Manganese is accumulated in cell wall mannoprotein according to Stehlik-Tomas, Zetic, Stanzer, Grba, & Vahcic (2004). They indicate that lower amounts of manganese are required than copper and zinc. Liang & Zhou (2007) studied the effect of metals on yeast at the mitochondrial level and observed that copper and manganese induce yeast apoptosis via different pathways but manganese was toxic from 4mM (22 mg/l). Manganese is reported by Jones and Greenfield (1984) to increase intracellular nitrogen content. Low FAN substrates such as sorghum, cassava starch and sugar depend more on manganese than a well balanced wort.

TABLE 1 NEAR HERE

Materials and methods

The data on metals in figures 1 & 2 was gathered from full scale (2000 Hl) fermentations after conventional wort extraction in brewhouse. The wort was prepared from 75% malt with 25% milled barley. The wort was pitched with S. cerevisiae from the brewery culture collection. Yeast was collected by centrifugation after the wort had reached its attenuation limit. Samples of the yeast were taken and frozen so that all metals could be measured in a single session to avoid any variations in technique. Metals in table 1 were measured by an accredited contract laboratory (Mountainheath). The results were expressed as yeast dry weight. Data shown in figures 3 & 4 was derived from fermentations in lager wort made with sorghum mashed with alpha amylase and protease fermented in 5 litre glass tubes. The wort was supplemented with the additions as described on the graphs and held at 20 degrees in a constant temperature room. Gravity was measured using a Paar densitometer. The yeast was S. pastorianus from the brewery culture collection.

The fermentations in figure 5 contained the following ingredients by extract contribution sorghum 62% and wheat 15% mashed in with alpha amylase and protease from Kerry Biosciences and high maltose syrup 23% added to the kettle (Cargill C sweet M01511 consisting of 49% maltose, 15% maltotriose and 3% glucose). Salts 0.2mg/l zinc,0.1 parts per million (mg/l) magnesium, $CaCl_2$ 600 mg/l, $CaSO_4$ 200 mg/l, $(NH3)_2PO_4$ 540 mg/l were added to the kettle. Carageenan kettle finings were used to coagulate the trub in kettle. The yeast was S. pastorianus from the brewery culture collection.

Data in figure 6 was derived from 1Hl glass tube fermentations containing high maltose syrup (Cargill C sweet M01511) consisting of 49% maltose, 15% maltotriose and 3% glucose). A salts mixture containing 0.2 mg/l Mn, 0.2 mg/l Zn, 4000 mg/l Lallemand Fermaid, 4000 mg/l $(NH3)_2PO_4$, 2000 mg/l $CaSO_4$, 500 mg/l $MgSO_4$. Amyloglucosisdase was added to make the limit dextrins in the high maltose feedstock more available. The yeast was Lallemand Thermosacc active dried yeast. Urea was supplemented at 300 mg/l. The fermentations were circulated to overcome any potential yeast flocculation.

All samples were frozen and analysed at the same time so that analytical error was minimized. Poreda, Antkiewicz, Tuszynski & Malgorzata (2009) observed that when biomass was deficient, cells retained their calcium through the fermentation while with greater biomass calcium was liberated emphasizing the importance of consistency when taking measurements of metal content of yeast. In this work care was taken to harvest all yeast for analysis at centrifugation after attenuation.

Results and Discussion

In studies on beer flavor, Zufall & Tyrell (2008) noted the greater influence of manganese compared with iron on beer flavor because it persisted in the beer after fermentation. This implied that iron was taken up by yeast while manganese remained. In this study figure 1 shows that manganese was accumulated while iron varied but did not show a trend. In industrial practice both iron and manganese are commonly precipitated by tannic acid in brewing to improve shelf life. If this happens in the brewhouse metals will not be available to the yeast. Natural tannins could have similar effects on concentration of metals. Effects on flavour and shelf life were not considered in this work. The flavor outcome observed in this study was a 40% increase in ethyl hexanoate. Other esters, for example, iso amylacetate and ethyl acetate did not increase.

Donhauser, Wagner, Schneider, (1985) reported faster fermentation when added manganese was increased in wort from 0.1-0.2 mg/litre to 0.16-0.27 mg/litre with no significant increase in by products. The faster fermentations described in figure 1 concur with this observation but manganese is shown to accumulate in the yeast cells as generations progress without supplementation of the wort.

FIGURE 1 AND FIGURE 2 NEAR HERE

Results in figure 2 indicate that the speed of fermentation increases when manganese accumulates in the yeast cells under the circumstances of the test. Ethyl hexanoate levels in resultant beer were significantly higher, changing the flavour. However, the grist consisted of barley and malt and no FAN deficiency was suspected.

Helin & Slaughter (1977) indicated the minimum levels of zinc (0.2.mg/l) and manganese (0.01 mg/l) required for fermentation and shows the interaction between zinc and manganese. The fermentations in figure 2 had been supplemented at these recommended levels but the effect of accumulation is to speed fermentation and produce additional ethyl hexanoate. This indicates that the cells are capable of using more manganese than supplied in the wort. Similar to this study Lentini , Jones, Wheatcroft, Lim, Fox, Hawthorne & Kavanagh (1981) observed increased levels of manganese in seven serial pitchings of yeast.

FIGURE 3 AND FIGURE 4 NEAR HERE

Dombek & Ingram (1986) suggested that the enhanced beneficial effect of complex nutrients could be due to correction of a simple ionic deficiency such as magnesium. He indicated that additional magnesium increased cell growth and retained fermentative activity to give faster fermentations with high yield. While this observation applies to complex materials it is unlikely to be the source of the positive effect found for urea shown in figure 6. Nitrogen supplementation by soya peptides can have a beneficial effect. The positive effect might be due to enzymes present in unheated varieties of peptide preparations from some sources. Di ammonium phosphate is the most effective of the simple nitrogen salts. In this study some preparations of di-ammonium phosphate were found to have a strong odour of ammonia which persisted into the finished beer. Kitagawa S, Mukai, Furukawa, Adachi, & Lefuji, (2008) reported on the effect of soy peptides with maltose as carbon source. They showed that increased beta phenylethanol was produced due to higher phenalanine present as dipeptide.

FIGURE 5 and FIGURE 6 NEAR HERE

Conclusion

The work in this paper emphasizes the difficulty in transferring precise, controlled laboratory data into the brewery. Understanding the complex interactions of ions and nitrogen sources will facilitate fermentation of simple sugars with minimal additions to produce new products economically. Super high gravity fermentations depend on the supplements added and these control attenuation and alcohol yield. Fermentation will not achieve attenuation without perfect nutrient status. Control of flavour for product consistency also depends on metallic ions in the wort. Excessively fast fermentations have the disadvantages of lower pH, excessive fobbing, change in flavour and the yeast harvested may perform differently in subsequent fermentations. This paper indicates that manganese needs to be controlled for fermentation consistency and flavor consistency. Since the initial concentration of manganese can be augmented within the yeast cell by accumulation the yeast must be freshly propagated at frequent intervals as is traditional practice to keep fermentations consistent.

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Table 1 Metal levels in malt and barley

Mg/kg	Cd	Pb	Mn	Cu	Zn	Fe	Ni	Mg	As	Al	Co	Sn
Comex Barley 17/5/07 mixed varieties	<0.25	2	7.6	4.3	13.2	69	0.5	1010	<0.25	1.06	<0.25	<0.25
Comex Barley 22/8/07 mixed varieties	<0.25	4	7.9	4.7	21.2	69	<0.5	1090	<0.25	2.57	<0.25	<0.25
Glanbia Barley 19/2/07 mixed varieties	<0.25	4	9.4	4.8	19.7	67	0.6	1010	<0.25	2.35	<0.25	<0.25
Glanbia Barley 30/8/07 mixed varieties	<0.25	3	11.1	5.1	16.1	60	0.6	901	<0.25	0.7	<0.25	<0.25
Saloon Barley 2005 crop	<0.25	4	10.4	6.6	25.1	78	<0.5	1160	<0.25	0.98	<0.25	<0.25
Cellar Barley 2005 crop	<0.25	4	11.2	7.3	21.7	74	<0.5	889	<0.25	2.8	<0.25	<0.25
Optic Barley 2005 crop	<0.25	5	17	7.1	23.8	128	<0.5	987	<0.25	2.47	<0.25	<0.25
Eunova Barley 2006 crop	<0.25	4	7.8	5.3	21.4	91	<0.5	1010	<0.25	0.62	<0.25	<0.25
Publican barley 2006 crop	<0.25	25	7.7	6.5	24.4	41	<0.5	971	<0.25	0.72	<0.25	<0.25
Fractal barley 2006 crop	<0.25	20	8.6	4.9	19.6	43	1.1	931	<0.25	1.78	<0.25	<0.25
Minch mixed malt 22/8/07	<0.25	13	6.9	4.2	12.5	55	0.5	1060	<0.25	0.74	<0.25	<0.25
Cork mixed malt 21/8/07	<0.25	14	7.5	3.4	13.8	56	<0.5	1170	<0.25	0.54	<0.25	<0.25
Minch mixed malt 17/5/07	<0.25	11	6.6	3.2	11.1	50	0.5	1150	<0.25	0.53	<0.25	<0.25
Minch mixed malt 19/2/07	<0.25	18	8.9	5.4	19.3	66	1.4	1160	<0.25	0.74	<0.25	<0.25
Optic Malt 17/4/07	<0.25	<1	8.8	2.6	14.5	31	<0.5		<0.5	0.59	<0.5	<0.5
Prestige Malt 7/4/07	<0.25	<1	8.7	2.2	11.9	26	<0.5		<0.5	0.55	<0.5	<0.5
Cocktail Malt 7/4/07	<0.25	1.8	7.7	2.3	15.6	35	<0.5		<0.5	0.6	<0.5	<0.5
Sebastian Malt 7/4/07	<0.25	<1	7	3	14.7	30	<0.5		<0.5	0.83	<0.5	<0.5

Figure 1 Metal accumulation with yeast generation

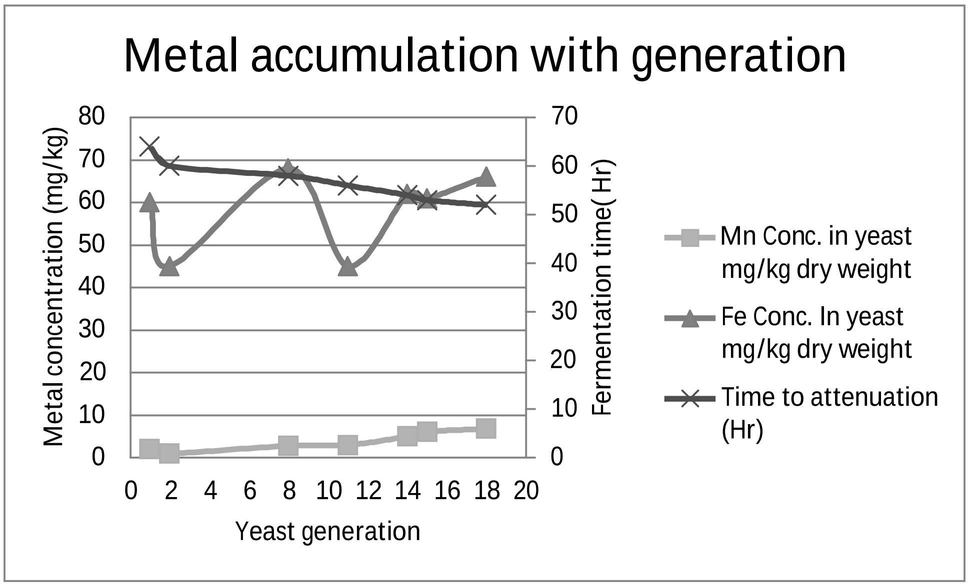

Figure 2 Ethyl hexanoate increase with generation

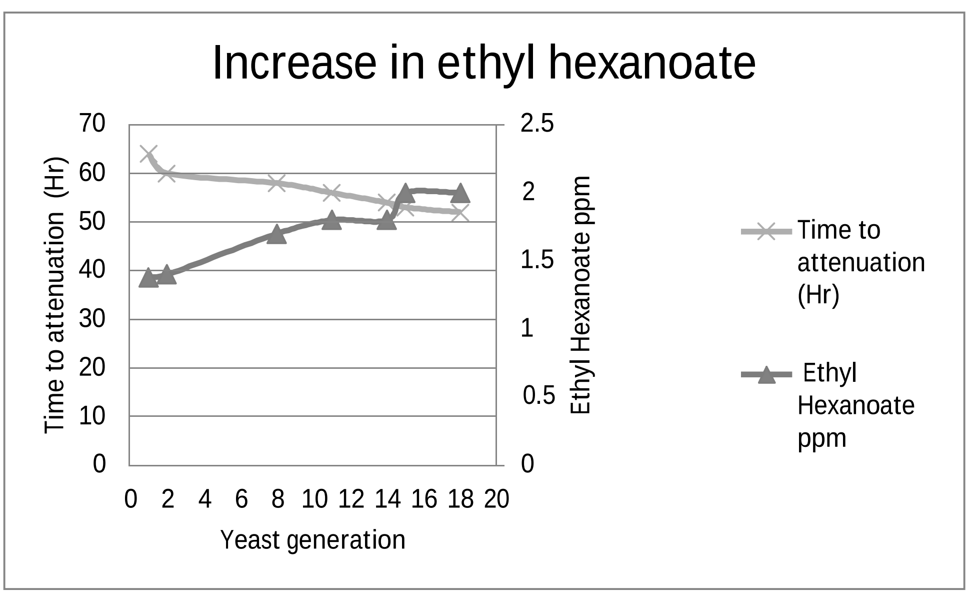

Figure 3 Local raw materials lager fermentation

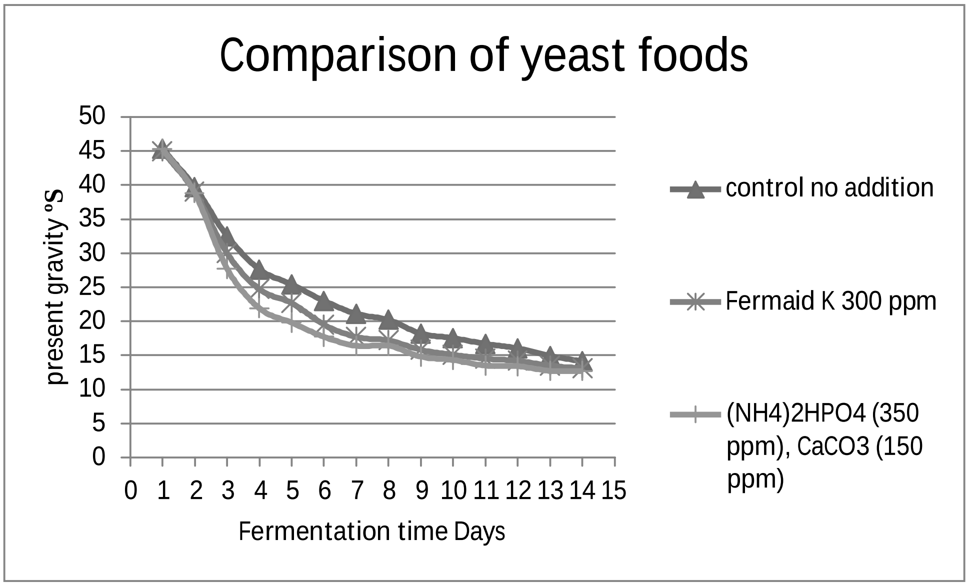

Figure 4 FAN supplements

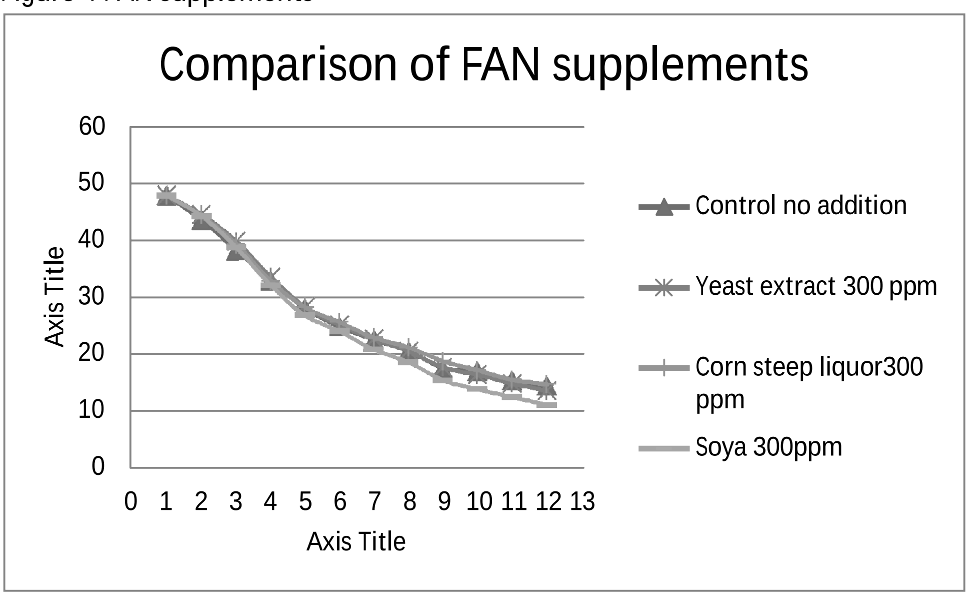

Figure 5 Comparison of yeast foods 2

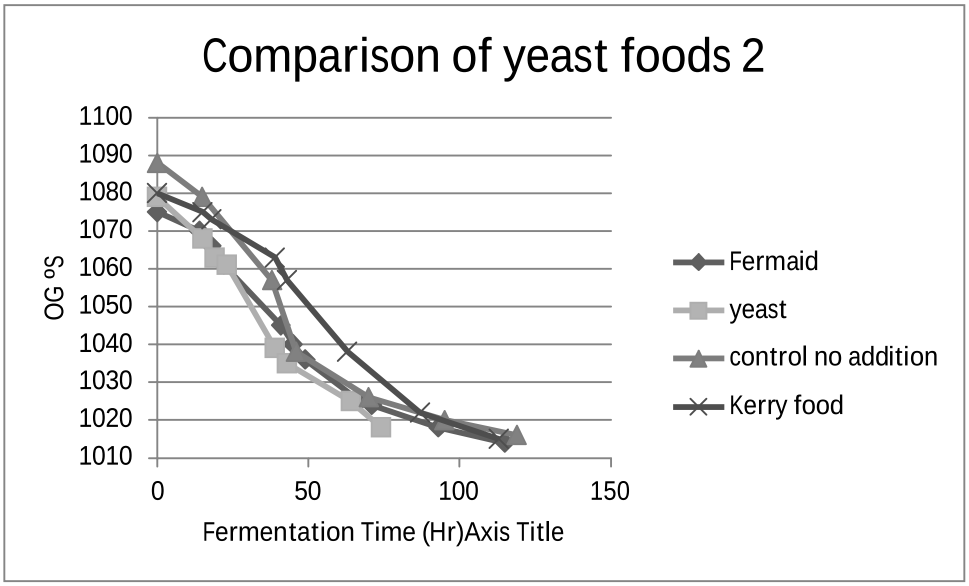

Figure 6 Effect of urea