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Optimisation of a Fully Defined Medium for Yeast Fermentation Studies

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Summary

It is known that there can be significant batch to batch variation in the chemical composition of brewery worts. Thus, wort does not provide a reproducible substrate for laboratory experiments when investigating yeast behaviour such as fermentation profile, uptake of nutrients and the formation of by-products.

Experiments were devised to optimise a fully defined medium which reflected the composition of industrially produced wort. The aim was to provide a reproducible medium for laboratory studies which was also comparable to production wort in supporting yeast growth and fermentation.

A mixture of amino acids, carbohydrates, vitamins and trace elements was developed based on analyses of brewery worts. This medium proved to be an excellent fermentation substrate for lager yeast giving results similar to those obtained with wort. Ale yeast could not fully attenuate the defined medium but otherwise produced results typical of wort fermentation.

Keywords: defined medium, wort fermentation, yeast, esters, carbohydrates

Introduction

As part of a stringent yeast management policy, it is desirable to define characteristic profiles for each production yeast strain. Typical profile components are laboratory scale fermentation of a standard medium, microbiological aspects (cell size and shape, colony morphology) and yeast classification tests (tullo, adhesion, sedimentation rate, head formation and carbohydrate utilisation). This enables monitoring of possible variations in yeast characteristics both in long term storage at source and in the production environment where the yeast is subjected to a number of selective pressures.

There can be significant batch to batch variation in the exact composition of brewery wort produced to the same recipe at different times. Thus, brewery worts do not provide the entirely reproducible substrate required for yeast profiling through small scale laboratory investigations of yeast behaviour, uptake of nutrients, ester and higher-alcohol production and the formation of by-products. A number of groups have made use of complex, chemically-defined »synthetic worts«: Suihko *et al. (1)* used a synthetic medium to investigate yeast sugar uptake and beer quality; Lee and Prentice *(2)* examined the utilisation of nucleosides and nucleobases by lager yeast; Evans and Hall *(3)* studied foams and antifoams and Wainwright *(4)* employed synthetic wort when investigating hydrogen sulphide production.

In this paper, the authors report experiments carried out to modify and optimise a fully defined medium, first proposed by Thompson *et al. (5)* to provide a reproducible »synthetic wort« which reflected brewery wort in composition of the essential nutrients required for yeast growth. The medium and brewery wort were fermented in parallel by ale and lager yeast production strains.

Materials and Methods

Bottom fermenting ale and lager yeast strains currently used by Scottish Courage Brewing Ltd. were chosen for this study. Lager or ale wort from full production scale was employed in the laboratory experiments. Bacto yeast nitrogen base w/o amino acids and ammonium sulphate was obtained from Difco (0335-15-9). Amino ac-

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ids, carbohydrates and other general laboratory reagents were obtained from Sigma-Aldrich.

Fermentations were carried out using EBC tubes *(6)*. The initial gravity of both wort and defined media were 1055 ° Saccharin. Brewery yeast slurries were always acid washed (2% volume fraction, orthophosphoric acid, pH = 2.1–2.3, 4 °C, 2–4 h) prior to use as inoculum. The inoculation rates for both lager and ale yeast strains were 2.0×10^7 viable cells mL^{-1}. Ale fermentations were carried out at 20 °C and lager fermentations at 15 °C, both followed by chilling to 4 °C to separate the yeast. Fermentations were carried out either in duplicate or in triplicate.

Defined medium was prepared by supplementing YNB w/o amino acids and ammonium sulphate with a mixture of fermentable sugars to give an initial gravity of 1055°. The ratio of the various carbohydrates (Table 1) was based on those generally found in brewery worts (Table 2). The assimilable nitrogen was supplied as a mixture of amino acids and ammonium sulphate (Table 3) which resulted in a free amino nitrogen (FAN) of 153 mg L^{-1} in the defined medium. For lager fermentation, the defined medium was further supplemented with (g L^{-1}) citric acid (0.625) and $CaSO_4 \cdot 2H_2O$ (0.215). The pH of the medium, prior to carbohydrate addition, was adjusted to 5.2 using NaOH (1 M) prior to sterilisation through pasteurisation (60 °C; 30 min). This was mixed with a sterile (121 °C, 20 min) solution of carbohydrates prior to inoculation. Wort was stored sterile (121 °C, 20 min), diluted to 1055° and supplemented with $ZnSO_4 \cdot 7H_2O$ (0.7 mg L^{-1}) prior to use. Both defined medium and wort were aerated (sterile air; 20 min) through gas distribution tubes prior to inoculation.

Samples were withdrawn periodically from EBC tubes for immediate yeast cell concentration and viability determination *(6)*. The clarified supernatants (2000 g; 10 min) of these samples were subjected to specific gravity and pH measurements. The specific gravity of samples were measured using a DMA 55 calculating density meter (Anton Paar). A pH probe (Orion) was employed for pH measurement. End of fermentation samples, free of the bulk of yeast population, were stored at –18 °C until analysed for headspace components, total vicinal diketones (TVD), and FAN.

Carbohydrates were measured by HPLC, volatile headspace components and TVD (diacetyl and 2,3-pentanedione) by headspace gas injection GC and FAN by Kjeldahl digestion. The methods adopted were modifications of the respective methods as recommended in the IOB Methods of Analysis *(6)*.

Table 1. Carbohydrate composition of defined medium

Carbohydrate	Amount added g L^{-1}
Glucose	10.4 g
Fructose	4.6 g
Sucrose	3.5 g
Maltose	115.5 g
Total	134 g

The grade of maltose used (Sigma grade II) contained up to 7% maltotriose, which was appropriate to a model of brewery wort.

Table 2. Typical carbohydrate composition in brewery lager wort (1055 °)

Component	Concentration g L^{-1}
Arabinose	<0.1
Glucose	8.30
Fructose	3.40
Sucrose	2.70
Maltose	94.20
Maltotriose	28.90
Maltotetrose	3.00

Table 3. Nitrogenous compounds composition of defined medium

Amino acids	Concentration mg L^{-1}
L-Aspartic acid	67.50
L-Threonine	46.80
L-Serine	37.50
L-Asparagine	128.60
L-Glutamine	5.20
L-Glutamic acid	77.80
L-Proline	272.90
Glycine	28.40
L-Alanine	88.40
L-Valine	93.60
L-Methionine	23.40
L-Isoleucine	49.60
L-Leucine	121.80
L-Tyrosine	80.20
L-Phenylalanine	95.60
L-Tryptophane	42.30
L-Lysine hydrochloride	112.20
L-Histidine hydrochloride	50.90
L-Arginine hydrochloride	138.40
Ammonium sulfate	130.70

From Thompson *et al. (5)*

Results

The initial set of fermentations, carried out with ale yeast, demonstrated the poor buffering capacity of the defined medium. The final pH of the culture was 2.97 and the viability of the cropped yeast was 57%. Flocculation occurred inefficiently.

In an attempt to alleviate the problems of poor buffering capacity and poor yeast flocculation the defined medium was supplemented with citric acid (0.63 or 1.26 g L^{-1}) and calcium sulphate (0.22 g L^{-1}). Using the higher concentration of citric acid in conjunction with calcium resulted in suitable buffering capacity of the medium and satisfactory flocculation characteristics of yeast. The final pH of the culture was 3.97 and the viability of the cropped yeast was 68%. Parallel fermentations using ale wort resulted in a final pH of 3.74 and the cropped yeast exhibited a viability of 67%. The TVD profile of the fermentations carried out using defined medium and wort closely followed each other (Fig. 1a). At the point when cooling was applied (100 h fermentation time) the gravity of the ale yeast fermented wort and defined medium were 1008° and 1022° respectively (Fig. 1b). The residual

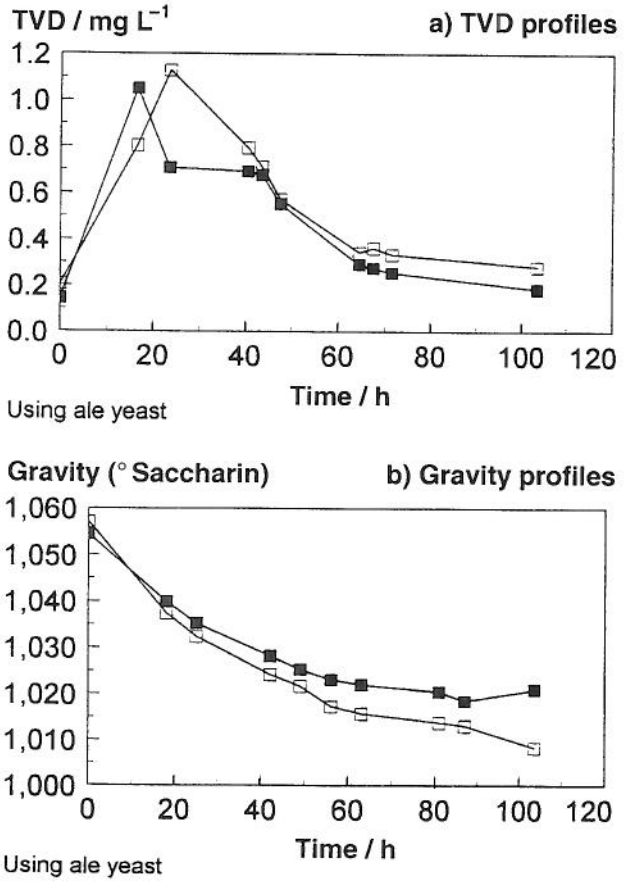

Fig. 1. Fermentation of defined medium and ale wort by yeast (Defined medium ■, Ale wort □)

carbohydrate concentrations of these were determined (Table 4). The formation of a yeast head and subsequent fermentation and sedimentation of the yeast was delayed by approximately 20 hours in the synthetic wort fermentations.

Fermentation of lager wort and defined medium by lager yeast were compared in triplicate. The attenuation profiles obtained with both media matched each other closely (Fig. 2a). The PG of all cultures fell below 1010° after approximately 65 h incubation, however, the composition of the residual carbohydrates in the two media were markedly different (Table 5). The pH of the fermented defined medium remained slightly higher than that of the lager wort throughout the fermentation (Fig. 2b). The TVD concentration profiles obtained with both media were very similar (Fig. 2c) as were the suspended cell concentration profiles (Fig. 2d). The viability of yeast in all cultures remained above 90% throughout the fermentation period.

The concentrations of the headspace components measured at the end of the experiment in fermented wort and defined medium were the same within the accuracy of the method (Table 6).

Discussion

The experiments with ale yeast were used to optimise the composition of defined medium *(5)*, allowing

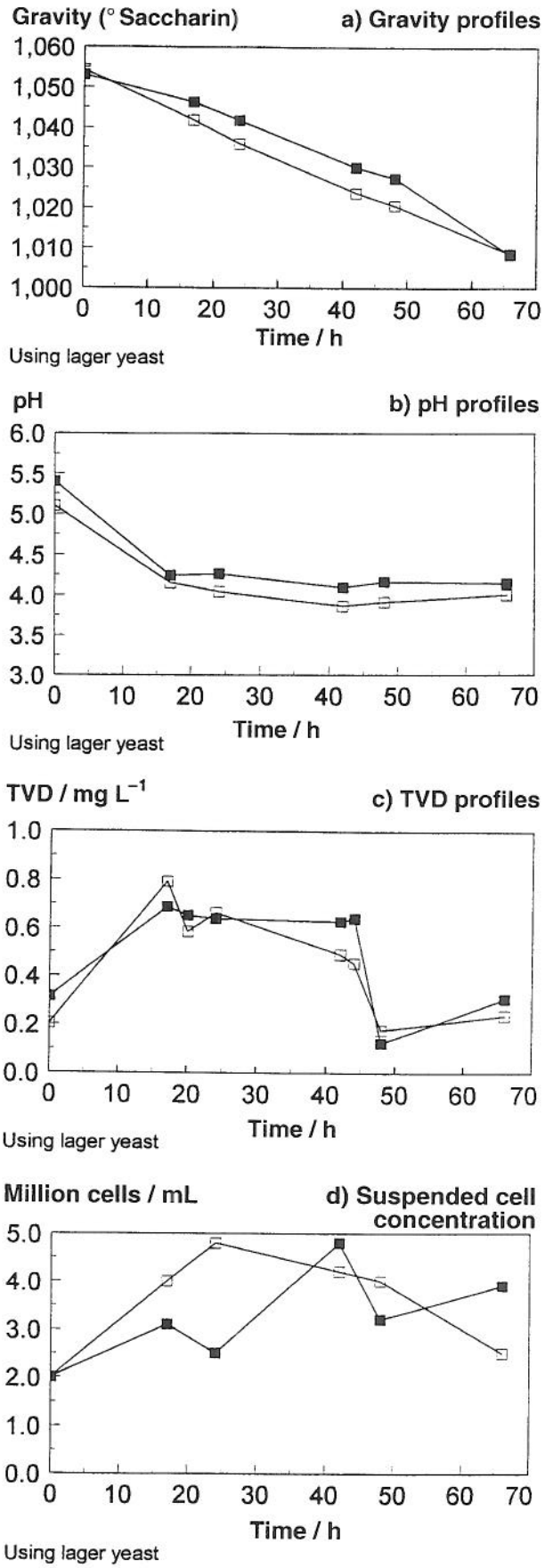

Fig. 2. Fermentation of defined medium and lager wort by yeast (Defined medium ■, Lager wort □)

Table 4. Residual concentration of carbohydrates after ale fermentation (g L^{-1})

	Arabinose	Glucose	Fructose	Sucrose	Maltose	Maltotriose
Initial concentration in defined medium	0.00	10.40	4.60	3.50	115.50	0.00
Final concentration in defined medium	0.00	3.30	0.00	0.00	71.20	3.30
Final concentration in ale wort	0.00	0.60	0.00	0.00	1.00	3.10

Table 5. Residual concentration of carbohydrates after lager fermentation (g L^{-1})

	Arabinose	Glucose	Fructose	Sucrose	Maltose	Maltotriose
Initial concentration in defined medium	0.00	10.40	4.60	3.50	115.50	0.00
Final concentration in defined medium	0.00	0.00	0.00	0.00	32.80	0.00
Final concentration in lager wort	0.00	0.00	0.00	0.00	1.00	0.80

Table 6. Headspace components at the end of lager fermenation

Headspace component	Defined medium mg L^{-1}	Lager wort mg L^{-1}
Ethylacetate	22.40	28.40
iso-Butyl acetate	0.10	0.14
Ethylbutyrate	0.20	0.20
n-Propanol	11.60	14.00
iso-Butanol	17.80	22.00
iso-Pentylacetate	2.88	3.04
iso-Pentanol	82.40	78.80
Ethylhexanoate	0.26	0.30
Ethyloctanoate	0.70	0.64

similar yeast flocculation behaviour in this medium to that observed in brewery wort. This was achieved by adjusting the buffering capacity of the medium to result in an end of fermentation pH similar to that of commercial beers (3.8–4.0). Wort contains high levels of proteins and peptides which provide a large buffering capacity and yeast flocculation is known to be affected by pH *(7)*. The other important factor for inducing flocculation in bottom fermenting yeast is calcium *(8,9)*. The concentration of the calcium in the defined medium was increased to that normally encountered in brewery worts (approximately 50 ppm of Ca^{2+}). This also resulted in a 2:1 ratio of Mg^{2+} to Ca^{2+} which is believed to be beneficial to yeast health *(10)*. The improved defined medium had a positive effect on the viability of the cropped yeast.

The partial attenuation of defined medium, when compared to wort, was caused by the incomplete uptake and metabolism of maltose, as apparent by the residual maltose in the medium at the end of fermentation. Lagunas *(11)* has reported that lack of nitrogen can result in the inactivation of sugar transport and that ale strains are more susceptible to this inactivation than lager strains. One explanation for the reduced fermentative capability of yeast in defined medium could have been the relatively low FAN content of this medium. Another possibility could include the presence of complex nutrients in wort which would result in increased fermentative activity of yeast.

The optimised defined medium proved an excellent substrate for fermentation by lager yeast. The behaviour of yeast, with respect to fermentation rate, yeast growth and viability, final attenuation, TVD production and removal, flocculation and production of the headspace components, was the same in both lager wort and defined medium. The fermented lager wort had the same PG (1008°) as that of the fermented defined medium. In the fermented wort the PG reflected the presence of dextrins. The residual fermentable carbohydrate concentration was lower in fermented wort than that present in fermented defined medium. This may have been in part due to the fact that no non-fermentable carbohydrate was present in the defined medium. It is conceivable that utilisation of the residual maltose would have continued at a relatively slow rate, had cooling not been applied. It is suggested that the decreased rate of fermentation could have been due, not to the lack of fermentable carbohydrate, but to oxygen depletion of the culture in the earlier stages of fermentation. It is generally accepted that in brewing fermentations it is oxygen starvation that often arrests yeast growth.

The similarity in the concentration of the headspace volatiles produced in defined medium and lager wort demonstrated that, at normal production concentrations, the production of these compounds is largely dependent on yeast strain. The experiments described here suggest that wort components other than carbohydrates and amino acids have little influence on the production of higher alcohols and esters commonly measured in beer. Amino acids *(12)*, wort gravity *(13)* and the composition of wort carbohydrates *(14)* have all been implicated in fusel oil production by yeast.

Conclusions

Clearly, the defined medium proved a much more suitable substrate for lager yeast than it did for ale yeast. Therefore, the defined medium in its present formulation satisfies all the requirements, outlined earlier, for lager yeast-profiling studies. It is hoped that with the very close genetic origin of lager yeast *(15)* this medium will not need further refinement when used in conjunction with other lager yeast strains. Work is currently being carried out in an effort to overcome the shortcomings of the defined medium when used in conjunction with ale yeast strains.

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Optimiranje potpuno definirane podloge za fermentaciju kvasca

Sažetak

Poznato je da postoje znatne razlike u kemijskom sastavu nehmeljenog piva. Stoga ta podloga ne sadržava istovjetne supstrate potrebne u laboratorijskim pokusima kada se istražuje ponašanje kvasca u procesu fermentacije, primanja hranjivih tvari i stvaranja nusproizvoda.

Pokusi su bili usmjereni na optimiranje potpuno definirane podloge koja bi odražavala sastav industrijski proizvedenog neprovrelog piva. Nastojala se pripraviti reproducibilna podloga za laboratorijska ispitivanja koja bi kao i nehmeljeno pivo omogućila rast kvasca i njegovu fermentaciju.

Na osnovi analize nehmeljenog piva pripravljena je smjesa aminokiselina, ugljikohidrata, vitamina i elemenata u tragovima. Ta se podloga pokazala odličnom za fermentaciju pivskog kvasca dajući slične rezultate kao što se dobivaju s nehmeljenim pivom. Kvasac engleskog piva ale nije mogao potpuno iskoristiti definiranu podlogu, ali je davao rezultate karakteristične za tipičnu fermentaciju piva.