The Use of Fatty Acid and Sterol Analyses as Quality Control Methods in the Brewing Industry

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ABSTRACT

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Fatty acid and sterol analyses were evaluated as alternative quality control methods to conventional differentiation and characterisation systems in the brewing industry. The presence of linoleic acid (18:2) in brewing yeast could be used to distinguish these from closely related yeast species. Furthermore, the absence of lanosterol and stigmasterol enabled differentiation of the brewing yeast from the rest of the closely related species tested. However, both fatty acid and sterol methods were not sensitive enough to detect mutants (variants) of brewing yeast. Conventional brewing identification tests proved sensitive enough to detect variants at low concentrations.

Key words: Brewing yeasts, fatty acids, quality control, sterols.

INTRODUCTION

Yeast identification using cellular long-chain fatty acids (FAs) spans over 30 years of research. Abel and co-workers[1] pioneered the application of FAs for taxonomic purposes by differentiating between various bacteria. Many researchers have since followed. In 1979, Gangopadhyay et al.[8] used cellular long-chain FAs to differentiate between 38 isolates of anamorphic genera of *Candida, Cryptococcus* and *Torulopsis.* In 1980, Gunasekaran and Hughes[9] used FA profiles to characterise 85 strains of *Candida* based on the presence or the absence of certain FAs. Successive studies on cellular FA composition of yeast from various taxa[10] showed that many yeast strains have characteristic FA profiles. Furthermore, the potential of this method for industrial application has been evaluated[3]. Subsequently, FA fingerprinting based on the presence or absence of especially linoleic (18:2) and linolenic (18:3) acids has been used for the characterisation of brewing yeasts[14]. It has also been reported that sterol composition together with FA profiles show promise as a taxonomic marker in fungi[13]. Both these lipid types are critical to membrane structure and function and are also associated with brewing yeast vitality and fermentation performance[16].

Apart from studies involving the chemotaxonomic status of FAs and sterols in differentiating yeasts from closely related species, it is also important to evaluate the sensitivity of these methods in detecting contamination by mutants. Mutation of brewing yeasts occurs spontaneously as a result of replication errors[12]. Mutants found in brewing are classified as variants and respiratory deficient mutants (RDs)[15]. Both mutant types are detrimental to beer quality i.e., causing stuck fermentation and producing off-flavours[6,11]. Consequently many breweries have strict specifications to combat this type of contamination[5]. In this study FA and sterol analyses were used to differentiate brewing yeast from closely related species. These techniques were also evaluated for sensitivity to detect contamination with mutants of brewing yeasts.

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Table I. *Saccharomyces* species and brewing strains used in this study[1].

Organism	Strain no.
Saccharomyces barnettii	CBS 5648**T**
Saccharomyces bayanus	CBS 0380**T**
Saccharomyces castellii	CBS 4309**T**
Saccharomyces cerevisiae	CBS 1171**NT**
Saccharomyces dairenensis	CBS 0421**T**
Saccharomyces exiguus	CBS 0379**T**
Saccharomyces kluyveri	CBS 3082**T**
Saccharomyces paradoxus	CBS 0406**T**
Saccharomyces pastorianus	CBS 1397**T**
Saccharomyces servazzii	CBS 4311**T**
Saccharomyces spencerorum	CBS 3019**T**
Saccharomyces transvaalensis	CBS 2186**T**
Saccharomyces unisporus	CBS 0398**T**
Brewing strain 1	UOFS Y-0494
Brewing strain 2	UOFS Y-0532

[1] CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **T** = Type strain; **NT** = New Type strain; UOFS = University of the Orange Free State, Bloemfontein, South Africa.

Table II. Results of the mutant mixed with the normal brewing yeast (UOFS Y-0494) to yield different concentrations of mutants (theoretical values) and the percentage actual mutants as counted from the Wallerstein Laboratory Nutrient medium (WLN) plates[1].

% Mutants (Theoretical values)	% Mutants (WLN)
0	1.7
2.7	4.4
3.7	5.4
4.7	3.5
5.7	3.9
6.7	6.5
7.7	8.5
8.7	6.2
9.7	11.8
100	94.1

[1] Standard error < 10%.

MATERIALS AND METHODS

Differentiation based on fatty acid and sterol analyses

Yeasts used. The yeasts used in this study are listed in Table I.

Preparation of brewing yeast mutant. Variants from the brewing strain UOFS Y-0494 were isolated on Wallerstein Laboratory Nutrient Medium (WLN) (Biolab, USA) plates after five days of incubation at 25°C. Mutant colonies are distinguished from normal brewing yeast on WLN since their colony morphology is markedly different (wrinkled, colony size smaller, edges not entire) and their colour is an intense blue[2].

Cultivation. Thirteen species of the genus *Saccharomyces* including two brewing yeasts and a selected stable variant were cultivated on YM agar slants[17]. All strains were then inoculated into 1 L conical flasks containing 400 mL of the following medium: YNB (Difco, USA), 6.7 g/L and glucose (Merck, Germany) 40.0 g/L. Flasks were incubated at 30°C 1°C, shaken at 160 rpm until stationary phase and then harvested by centrifugation at 8000 rpm for 20 min and washed with distilled water. The centrifuged cells were rapidly frozen, followed by freeze drying.

Lipid extraction. All lipids were extracted from freeze dried cells with the aid of chloroform/methanol (2:1, by vol)[7], dried to constant weight and then weighed.

Fatty acid analysis. The FA composition was determined after trans-esterification by the addition of trimethyl sulphonium hydroxide[4]. Fatty acid methyl esters were analysed by using a Hewlett Packard 5890 gas chromatograph equipped with a Supelcowax 10 capillary column (30 m × 0.53 mm) with nitrogen as carrier gas set at a flow rate of 4 mL/min. The inlet temperature was set at 180°C with the initial column temperature set at 120°C increasing at 3°C/min to 225°C. Following a 10 min isothermal period, temperature was increased at the same rate to a final temperature of 240°C. The FA peaks were detected using a flame ionisation detector set at 300°C. Peaks were identified using authentic standards supplied by Sigma (USA). All experiments were performed in triplicate.

Table III. The lipid composition of the brewing yeasts and 13 species of the genus *Saccharomyces*[1].

Organism		Total lipids (% w/w)	Fatty acids (% w/w)					
			16.0	**16.1**	**18.0**	**18.1**	**18:2**	**18:3**
Sacch. barnettii	CBS 5648**T**	2.6	12.5	54.9	4.4	28.2	-	-
Sacch. bayanus	CBS 0380**T**	4.9	23.5	45.7	6.4	24.4	-	-
Sacch.castellii	CBS 4309**T**	9.3	16.9	50.3	3.8	29.0	-	-
Sacch. cerevisiae	CBS 1171**NT**	6.3	13.6	44.1	7.1	35.2	-	-
Sacch. dairenensis	CBS 0421**T**	1.6	15.5	58.9	3.9	21.7	-	-
Sacch. exiguus	CBS 0379**T**	4.1	16.8	53.5	5.0	24.7	-	-
Sacch. kluyveri	CBS 3082**T**	3.9	14.1	40.9	4.7	33.8	4.4	2.1
Sacch. paradoxus	CBS 0406**T**	5.3	9.9	51.7	5.0	33.4	-	-
Sacch. pastorianus	CBS 1397**T**	3.1	11.9	54.8	3.3	30.0	-	-
Sacch. servazzii	CBS 4311**T**	2.3	15.1	56.5	8.6	19.8	-	-
Sacch. spencerorum	CBS 3019**T**	4.4	16.1	51.7	4.9	27.3	-	-
Sacch. transvaalensis	CBS 2186**T**	1.0	10.2	59.7	2.6	27.5	-	-
Sacch. unisporus	CBS 0398**T**	3.2	14.5	61.7	5.9	17.9	-	-
Brewing strain 1	UOFS Y-0494	4.8	9.7	58.0	2.8	28.9	0.6	-
Brewing strain 2	UOFS Y-0532	3.9	7.9	53.2	4.4	34.2	0.3	-
Mutant		5.1	9.7	58.0	2.6	29.1	0.6	

[1] CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **T** = Type strain; **NT** = New Type strain; UOFS = University of the Orange Free State, Bloemfontein, South Africa. Standard error < 10%.

Table IV. Sterol composition of the brewing yeasts and 13 species of the genus *Saccharomyces*[1].

Organism		Ergosterol mg/g	Lanosterol mg/g	Stigmasterol mg/g	Squalene mg/g
Sacch. barnettii	CBS 5648**T**	367.3	45.8	109.0	25.0
Sacch. bayanus	CBS 0380**T**	217.5	35.0	628.0	225.0
Sacch.castellii	CBS 4309**T**	357.0	-	-	9.0
Sacch. cerevisiae	CBS 1171**NT**	452.5	152.5	405.5	159.0
Sacch. dairenensis	CBS 0421**T**	158.5	40.0	182.5	32.0
Sacch. exiguus	CBS 0379**T**	422.5	-	-	32.0
Sacch. kluyveri	CBS 3082**T**	367.5	42.0	86.5	9.0
Sacch. paradoxus	CBS 0406**T**	443.0	61.5	551.0	24.5
Sacch. pastorianus	CBS 1397**T**	610.0	134.0	606.0	102.0
Sacch. servazzii	CBS 4311**T**	239.5	237.0	154.0	9.0
Sacch. spencerorum	CBS 3019**T**	544.8	98.0	332.0	122.9
Sacch. transvaalensis	CBS 2186**T**	273.8	109.2	145.5	28.6
Sacch. unisporus	CBS 0398**T**	454.0	132.5	227.0	2.0
Brewing strain 1	UOFS Y-0494	398.3	37.9	-	14.9
Brewing strain 2	UOFS Y-0532	367.8	46.3	-	72.1
Mutant		342.2	30.6	-	11.9

[1] CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **T** = Type strain; **NT** = New Type strain; UOFS = University of the Orange Free State, Bloemfontein, South Africa. Standard error < 10%.

Sterol analysis. Hydrolysis: Freeze dried cells of each strain were hydrolysed as described by Rencken et al.[16]. In short, KOH dissolved in methanol:ethanol:dH_2O (700:-315:15, by vol) were added to pre-weighed freeze dried yeast biomass. A further 2 mL ethanol was added and the mixture was heated to 80°C for 90 min in sealed screw cap glass tubes (Schott, USA) while shaken gently and then cooled to room temperature.

Extraction: Sterols were extracted with pentane and stored overnight at 4°C. After evaporation using gaseous nitrogen, ethanol was added to the extract which was also left overnight at 4°C. The suspension was filtered and injected into a HPLC (Beckman System Gold, 2347). Separations were carried out on a Spherisorb C18 reverse phase column (25 cm × 0.46 cm, 5 µm diameter) using a mixture of ethanol:methanol:water, (100:850:50, by vol) as solvent phase at a flow rate of 1 mL/min. A Lambda-Max (Waters, Milford, MA, USA) Model 480 LC spectrophotometer was used to measure ergosterol at 282 nm and lanosterol, stigmasterol and squalene at 210 nm. Sterols present in each sample analysed were calculated as the ratio of the sterol peak area to the internal standard peak area in the sample chromatogram and in the chromatogram of a standard solution. All experiments were performed in triplicate.

Sensitivity of conventional identification tests

Yeasts used. The brewing yeast strain UOFS Y-0494 held in the University of the Free State Culture Collection (University of the Free State, Bloemfontein, South Africa) and the derived mutant isolated as described (see Preparation of brewing yeast mutant), were investigated.

Culturing. The brewing yeast UOFS Y-0494 and respective variant were grown in 1000 mL and 400 mL YM[17] medium contained in 5 L and 1 L Erlenmeyer flasks respectively, at 25°C for 12 h while shaking at 160 rpm. After harvesting as described before, mixtures were established by adding the selected stable variant to the brewing yeast to yield different concentrations, ranging from 0 % to 100 % (w/w) (theoretical values- see Table II).

Analysis. A 1 mL aliquot was then drawn from each mixture and analysed for contaminants according to brewery protocol[2] using WLN plates. All experiments were conducted in triplicate.

Reagents used. All reagents used were of high purity and obtained from reputable dealers.

RESULTS AND DISCUSSION

According to the results in Table III, brewing and related yeasts are characterised by the presence of mainly 16:0, 16:1, 18:0 and 18:1, while traces of 18:2 could be detected in the brewing strains and mutant. On the basis of this it is possible to differentiate the brewing strains and mutant from all related yeasts except *S. kluyveri.* The latter could clearly be distinguished from the rest of the species tested due to the presence of 18:2 and 18:3 FAs. Consequently, 18:2 and 18:3 may be used as an identification marker for the brewing yeasts tested. It is however important to note that no significant difference between the brewing strain (UOFS Y-0494) and derived mutant could be found. Other mutants tested in the same way gave similar results (results not shown). This renders FA profiles not sensitive enough to detect mutants formed during the brewing process.

Most organisms contained relatively high amounts of ergosterol, while varying amounts of lanosterol, stigmasterol and squalene were detected (Table IV). *S. castellii* and *S. exiguus* did not accumulate any lanosterol while *S. castellii, S. exiguus* and the brewing strains with mutant did not accumulate stigmasterol under the conditions studied. Although sometimes in low concentration, all yeasts studied could produce squalene. Consequently, the ability to produce lanosterol and stigmasterol can be used as marker to differentiate the closely related yeasts studied from the brewing strains and mutant. *S. pastorianus* produced generally higher amounts of sterols compared to the rest of the species (1452 mg/g in total). Again no significant difference could be detected between the brewing strain (UOFS Y-0494) and the derived mutant (Table IV).

The results illustrated in Table II showed that the conventional methods[2] as applied in the breweries, were sensitive enough to detect a mutant at different theoretical percentages i.e. 0% to 100% (correlation coefficient: r = 0.89045). Further research should be performed on other mutants derived in the same way to evaluate these methods for possible use in the brewing industry.

CONCLUSIONS

Although FA and sterol analyses could be used to differentiate qualitatively between the brewing strains (similar profiles) and closely related yeasts, they could not distinguish the mutant studied from the normal brewing strain from which it was derived. These data show that the conventional identification system used in the brewing industry is superior to the two chemotaxonomic methods tested.

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REFERENCES

1. Abel, K., De Schmertzing, H. and Peterson, J. L., *Journal of Bacteriology*, 1963, **85,** 1039.
2. Analysis Committee of the Institute of Brewing. Institute of Brewing:UK, 1997, 21.16, 21.17.
3. Botha, A. and Kock, J. L. F., *International Journal of Food Microbiology*, 1993, **19,** 39.
4. Butte, W., *Journal of Chromatography*, 1983, **26,** 1142.
5. de Angelo, J. and Siebert, K. J., *Journal of the American Society of Brewing Chemists*, 1987, **45,** 135.
6. Ernandes, J. R., Williams, J. W., Russell, I. and Stewart, G.G., *Journal of American Society of Brewing Chemists*, 1993, **51**, 16.
7. Folch, J., Lees, M. and Stanley, G. H. S., *Journal of Biological Chemistry*, 1957, **226,** 497.
8. Gangopadhyay, P. K., Thadepalli, H., Roy, I. and Ansari, A., *Journal of Infectious Diseases*, 1979, **40,** 952.
9. Gunasekaran, M. and Hughes, W. T., *Mycologia,* 1980, **72,** 505.
10. Kock, J. L. F. and Botha, A., Fatty acids in fungal taxonomy. In: Chemical Fungal Taxonomy, J. C. Frisvad, P. D. Bridge and D.K. Arora, Eds., London: Academic Press, 1998.
11. Lodolo, J. E., The effect of oxygen on the fermentation ability of *Saccharomyces cerevisiae* during high gravity wort fermentations. Ph.D. Thesis. University of Stellenbosch, 1999.

12. Monaghan, R. L., Gagiliardi, M. M. and Streicher, S. L., Culture preservation and inoculum development. In: Manual of Industrial Microbiology and Biotechnology. 2nd ed. A. L. Demain and J. E. Davies, Eds., ASM Press: Washington, D. C., 1999.
13. Müller, M. M., Kontola, R. and Kitunen, V., *Mycological Research*, 1994, **98,** 93.
14. Oosthuizen, A., Kock, J. L. F., Botes, P. J. and Lategan, P. M., *Applied Microbiological Biotechnology*, 1987, **26,** 55.
15. Priest, F. G., In: An Introduction to Brewing Science and Technology. Part 11, The Institute of Brewing: London, 1981.
16. Rencken, I., Fleming, V., Meijering, I. and Axcell, B., *Journal of Chromatographic Science*, 1995, **33,** 525.
17. Wickerham, L. J., *Technology Bulletin*, 1951, 1029.

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