Evaluation of a Brazilian Fuel Alcohol Yeast Strain for Scotch Whisky Fermentations

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ABSTRACT

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Traditionally, distilling companies in Scotland have employed a very limited number of yeast strains in the production of alcohol for Scotch whiskies. Recent changes such as the decline in availability of brewers' yeast as a secondary yeast strain and the availability of yeast in different formats (e.g., dried and cream yeast as alternatives to compressed yeast) have promoted interest in alternative Scotch whisky distilling yeasts. In previous work, we investigated different strains of yeasts, specifically Brazilian yeasts which had been isolated from and used in fuel alcohol distilleries. One of the Brazilian yeasts (CAT 1) showed a comparable fermentation performance and superior stress tolerance compared with a standard commercial Scotch whisky distilling yeast (M Type). The Brazilian CAT 1 yeast isolate was further assessed in laboratory scale fermentations and subsequent new make spirit was subjected to sensory analyses. The spirits produced using the Brazilian strain had acceptable flavour profiles and exhibited no sensory characteristics that were atypical of Scotch whisky new make spirit. This study highlights the potential of exploiting yeast biodiversity in traditional Scotch whisky distillery fermentation processes.

Key words: alcohol fermentation, bioethanol, Brazil, malt wort, Scotch whisky, yeast.

INTRODUCTION

According to the legal definition of Scotch whisky as outlined in the Scotch Whisky Order (1990)[25] and the Scotch Whisky Act (1988)[24], to be called Scotch whisky, the spirit must be produced following fermentation only by addition of yeast[21]. This definition also complies with the European Community Council (1989) definition of whisky[12]. However, the particular species or strain of yeast is not specified in these regulations. Many changes have been made to production processes with the objective of improving the efficiency of whisky production and development of new spirits[26], but these developments have largely not extended to yeast, although recent changes such as the decline in availability of brewers' yeast as a secondary yeast strain and the availability of yeast in different formats (e.g., dried and cream yeast as alternatives to compressed yeast) have promoted interest in alternative Scotch whisky distilling yeasts. The choice of yeast strains in distillery fermentations is fundamental not only to obtain high ethanol yields, but also to contribute in a positive manner to flavour congeners. Ideally, such yeasts should also maintain viability and vitality when exposed to environmental stresses, such as high temperature, osmotic pressure and alcohol toxicity.

Yeasts employed for fuel alcohol production in Brazil, and Scotch whisky production in Scotland, are selected to perform fermentations under differing conditions (Table I).

Fuel ethanol production in Brazil occurs during a continuous sugar cane harvest season that takes place over a period of 200 days. The juice of the sugar cane (sucrose), or the residual molasses from sugar refining processes, is inoculated with yeasts to carry out rapid fermentations. Many Brazilian distilleries use semi-continuous modified Melle Boinot fermentation processes[1,35], which employ very high yeast cell concentrations and produce alcohol concentrations of between 6–11% (v/v) in very short fermentation times (between 6 to 10 h). After the end of each fermentation cycle, the yeast cells are separated from fermented medium (beer) and concentrated by centrifugation. The concentrated yeast receives a treatment with diluted sulphuric acid for one or two hours (at pH 2.0–2.5) to kill contaminant bacteria. After this acid treatment, the yeast is re-pitched into fermentation vessels. Meanwhile, the fermented liquor (beer) is distilled to produce anhydrous bioethanol for use as a supplement to internal combustion engine fuel.

In contrast, the main objective of Scotch whisky fermentations is to convert cereal wort (derived either from barley malt, in the case of malt whisky, or wheat or maize, in the case of grain whisky) to potable ethanol with the concomitant formation of carbon dioxide and minor fermentation metabolites (congeners). These congeners contribute to the organoleptic qualities of the final distilled product[14]. No external enzymes or additives are permitted to be used in the processing of cereals for the production of Scotch whisky.

Scotch whisky fermentations take longer than those for fuel alcohol fermentations, typically lasting for 2–5 days.

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Table I. Similarities and differences between Brazilian fuel alcohol and Scotch whisky fermentation processes[a].

Parameter	Brazilian fuel alcohol	Scotch malt and grain whisky
Yeasts employed	*Saccharomyces cerevisiae*. Baker's yeast at start (then indigenous yeasts predominate)	*Saccharomyces cerevisiae*. Specially selected distiller's strains
Main fermentable sugar	Sucrose from sugarcane juice or molasses. Yeasts employed must have good invertase activity and ferment glucose and fructose.	Maltose from barley malt in Scotch malt; barley malt plus wheat or maize in Scotch grain whiskies. Yeasts employed must have good maltase activity and also have ability to ferment maltotriose.
Yeast pitching rate	8–17% wet weight	$10–20 \times 10^6$ cells/mL
Yeast re-cycling	Yes	No
Fermentation temperature	30°C (and above)	Starts at ~20°C rises to ~32°C
Yeast acid-treatment	Yes	No
Final ethanol yield	6–11%(v/v)	8-10%(v/v)
Fermentation time	6–10 h	24-48 h
Lactic acid bacteria	Undesired throughout fermentation	Undesired at start, desired at end of fermentation
Wort gravity, pH	Variable (using sugarcane juice or molasses) to yield 8–11% (v/v) ethanol, pH 4–5	~1060 OG (15°Plato), pH 5

[a] Although there will be inter-distillery variations in both countries, the information is considered generally representative.

The major categories of flavour congeners produced by Scotch whisky yeasts during fermentation include: higher alcohols (e.g., n-propanol and iso-butanol), esters (e.g., ethyl acetate and iso-amyl-acetate), aldehydes and ketones (e.g., acetaldehyde, furfural and diacetyl), sulphur compounds (e.g., dimethyl sulphide), organic and fatty acids [7,8,34]. Scotch whisky distilling yeast must tolerate the osmotic stress of the initial sugar concentration in the wort and be capable of fermenting wort sugars (mainly maltose and maltotriose) to ensure maximum conversion of starch derived carbohydrates into alcohol. They should also have the ability to complete the fermentation to give a final wash alcohol content of at least 8–10% (v/v) ethanol. In addition, lack of flocculence, minimal foaming, and a good temperature tolerance to ensure rapid fermentation above 30°C are necessary yeast attributes for efficient performance in producing potable alcohol for distilled spirits such as Scotch whisky. These characteristics are also desired in fuel alcohol yeasts. In both the Brazilian and Scottish fermentation processes, the wort used is not sterilized and a wide variety of contaminant organisms (yeasts and bacteria (particularly lactic acid bacteria)) can compete with the distilling yeasts for fermentable sugars resulting in potential significant losses in alcohol production. In Brazil, lactic acid bacteria can seriously affect ethanol yields[1], but in Scotland these bacteria, which are kept under control by active yeast fermentation and are maintained at acceptable levels with modern plant hygiene techniques, can predominate later in the fermentation. These are considered to play important roles in development of final flavour characteristics of Scotch whisky[24,31].

Yeasts used in alcohol distilleries, whether in Brazil for bioethanol, or Scotland for whisky, are all strains of *Saccharomyces cerevisiae* (*S. cerevisiae*). However, there is great biodiversity in yeasts, including intra-species variability[32].

Some Brazilian yeast strains isolated from distillery environments can produce elevated levels of succinic acid and this represents a desired attribute for yeast selection for this application. This is because of the synergistic action between succinic acid and alcohol which acts to reduce bacterial contamination in alcoholic fermentations[1].

Many factors influence yields in alcohol distilleries including yeast ethanol tolerance, wort original gravity, yeast pitching rate, and cell viability. Ethanol tolerance in high gravity fermentations is a desirable distilling yeast trait and is influenced by plasma membrane composition, media composition, mode of substrate feeding, osmotic pressure, temperature, intracellular ethanol accumulation and bioproduct formation[13,15]. Alcohol exerts a strong inhibitory action on yeast cell growth and ethanol production, which for many ethanol production plants is limited to no more than 13% (v/v)[3], although nowadays, some beverage and fuel alcohol plants can operate at higher ethanol levels. Different yeast strains have different alcohol tolerances[2,23], but the potential to reach ethanol yields as high as 23% (v/v) is feasible[30].

Distilling yeasts are chosen because of key traits, including fast and complete fermentation, alcohol tolerance, and rapid growth in oxygen-limited environments[34]. Currently, two commercial strains of *S. cerevisiae* predominate in the Scotch whisky industry, namely M Type (Kerry Biosciences, Menstrie, Scotland) and Mauri Pinnacle, (Mauri Yeast Products, Hull, England). Such yeasts can be supplied in a variety of formats: as pressed cake, or slurry (cream), which are widely used, or in a dried form which can be useful in some circumstances, but is not used as extensively. These distilling yeasts have been specifically developed for use in whisky fermentations[19], but indigenous (distillery-resident) yeasts, such as those considered in this paper, also have potential for industrial exploitation for alcohol production[1,4,11].

Although the main driver for developing new yeast strains for Scotch whisky production is to maximise fermentation efficiency to give increased alcohol yields, the ability to consistently produce new make spirit of desirable quality is also of major importance.

The aim of this study was to carry out a detailed comparative evaluation of yeasts isolated from Brazilian fuel alcohol distilleries with a standard Scotch whisky yeast strain (M type). It was anticipated, given the more extreme conditions used for fuel alcohol production, that the Brazilian yeast strains would be more stress tolerant than their Scotch whisky counterparts. It was also deemed of significant interest to test their suitability for Scotch whisky fermentations where maltose, rather than sucrose, was the main fermentation substrate.

Preliminary studies[5,6] had been carried out previously on four *S. cerevisiae* yeast strains, PE 2, CAT 1 and VR 1

Table II. Relationship between wash final gravity and wash alcohol strength after 4 days fermentation (wort OG 1080° and temperature range 19–33°C) (Berbert de Amorim Neto[5]; Berbert de Amorim Neto et al.[6]).

Yeast	Final gravity (°)	Alcohol (% [v/v])
M Type	1000.0	10.41
CAT 1	1003.4	10.04
PE 2	1008.9	8.37
VR 1	1028.4	6.45
BG 1	1060.5	2.50

and BG 1 which were obtained from different Brazilian distilleries; Pedra, Catanduva, Vale do Rosário and Barra Grande. These yeasts are normally used alone or mixed with baker's yeasts as starter cultures by many Brazilian distilleries. Baker's yeast is very useful at the beginning of commercial fuel ethanol fermentations, when distilleries need several tons of yeast cells, but they are replaced after a relatively short period of time by wild yeasts from the industrial environment. Such indigenous acclimatised Brazilian distillery yeasts have been characterised by karyotyping[4], and exhibit good performance in molasses fermentations with regard to parameters such as rate of fermentation, yeast viability, low foam, low glycerol, lack of flocculation and ethanol and temperature tolerance.

The results of previous investigations into the performance of the four Brazilian yeast strains[5,6] (Table II) showed that the Brazilian yeasts, pitched into high gravity malt wort (1080°) and fermented over a temperature range of 19°C to 33°C, demonstrated large differences between yeast strains in terms of their fermentation performance. The study indicated that the Brazilian yeast, CAT 1 had performed well in test fermentations and was therefore selected for further investigation.

EXPERIMENTAL

Yeast strains investigated

The Brazilian yeasts were originally selected by Fermentec S/C Ltda (Piracicaba, Brazil), which is a company providing a consultancy service to many distilleries in Brazil and worldwide. The *S. cerevisiae* yeast, CAT 1 was obtained from the Catanduva distillery in Brazil. The Brazilian fuel ethanol yeast was revived from lyophilized ampoules in MYGP (malt extract 3 g/L, yeast extract 3 g/L, glucose 10 g/L, and peptone 5 g/L) prior to assessment of fermentation characteristics. The CAT 1 strain was compared with one of the standard commercial Scotch whisky yeast strains, M Type (Kerry Biosciences). Brazilian yeast strains were obtained as pure lyophilised cultures from suppliers and grown using standard propagation techniques. M Type yeast samples were obtained fresh from a local Scotch whisky grain distillery and propagated in a similar manner.

Laboratory fermentations

Malt wort was collected from a Scotch malt whisky distillery from the point just after wort cooling, prior to yeast addition. The collected wort was transferred to the laboratory, separated into suitable aliquots and stored frozen (-20°C) until required. Prior to use in laboratory fermentations, the wort (approximate OG 1080°) was defrosted and diluted with distilled water to the desired original gravity. Following standard practice for laboratory-scale Scotch whisky fermentations, which are not sterile, the wort was not autoclaved prior to use. Long term experience with routine sampling in this manner has indicated no significant issues with bacterial growth. The amount of yeast required to inoculate the fermentations was calculated and was equivalent to using caked (pressed) yeast at a standard laboratory pitching rate of 0.4% (w/v). Inoculum yeast cells were harvested by centrifugation, after 72 h growth in MYPG (30°C with shaking), before pitching into 500 mL round-bottom flasks containing 350 mL wort. Fermentation temperature programmes were based on standard Scotch whisky distillery profiles, used at the Scotch Whisky Research Institute, similar to that described in Berbert de Amorim Neto et al[6] and was set using a programmable water bath controller (Grant GR150). The initial setting temperature was 19°C and was raised in standard increments, to a final temperature of either 33°C (normal temperature profile) or 36°C (high temperature profile), which was reached after 30 h. These temperatures were maintained until the end of fermentation. In experiments where new make spirit was subjected to congener and sensory analyses, larger scale fermentations were performed by adding the yeast to 2 L of wort at the standard pitching rate (equivalent to 0.4% (w/v) of pressed yeast) using the same fermentation temperature programmes as described above.

Sugar fermentation tests

A 10× stock solution of Yeast Nitrogen Base (YNB) and sugar was prepared (6.7 g of YNB and 5 g of sugar in 100 mL of purified water) for each sugar to be tested. The sugars used in this work were glucose, fructose, sucrose, maltose, maltotriose, lactose, raffinose, galactose and melibiose. The solutions were mixed and filter sterilized using either a 0.22 μm or 0.45 μm membrane filter. The stock solutions were then stored at 2–8°C until required. The final growth medium was prepared by aseptically pipetting 0.5 mL of 10× stock solution into 4.5 mL sterile water and mixing thoroughly.

Suspensions of CAT 1 and M Type yeast were prepared by adding a loopful of yeast cells (from a pure culture) to 10 mL of sterile distilled water and mixing. Aliquots (10 μL) of yeast suspension were then used to inoculate a series of test tubes containing final growth medium. Cultures were mixed well and incubated at a temperature of 25, 30, 35 or 40°C for 1 week (168 h). Yeast cell growth was determined daily by measuring the turbidity of cultures using a spectrophotometer at a wavelength of 660 nm against a blank (where increase in turbidity represents yeast cell growth). All tests were carried out in duplicate.

Analyses of fermentations

Samples of fermented wash were collected at regular intervals during fermentation to determine wash specific gravity (SG), alcohol strength, pH and yeast viability.

Wash specific gravity. Fermented wash was filtered through 2V filter paper (Whatman) and the filtrate was collected and equilibrated in a water bath at 20°C before measuring specific gravity using a Paar DMA 5000 densitometer.

Alcohol strength. Fermented wash was gently distilled and the distillate was collected in a volumetric flask and equilibrated at 20°C. Distillate volume was adjusted accurately to 100 mL with distilled water then filtered through GF/A filter paper. Alcohol strength was determined using a Paar DMA 5000 densitometer and expressed as percentage alcohol (% v/v).

Wash pH. The pH of a small volume of unfiltered wash was measured using a Jenway 3310 pH meter and probe.

Yeast percentage viability. Yeast percentage viability was determined using a standard methylene blue method[16] where wash samples were diluted appropriately and mixed with methylene blue stain prior to counting with an improved Neubauer haemocytometer.

Distillations to assess spirit quality

Once the fermentations were complete, the wash samples were double distilled using glass stills containing 10 g of fresh copper wool in the lyne arm (to simulate pot stills in malt whisky distilleries). Distillation rate was controlled using a Bunsen burner and each wash still charge was 2,000 mL of wash producing 700 mL of distillate (low wines). The distillation time was approximately 3.5 h. The low wines were then redistilled and three distillate fractions were collected: firstly, the *foreshots* (10 mL from distillation); secondly, the *new make spirit* (155 mL); and finally, the *feints* (235 mL). The time taken to collect the new make spirit fraction was approximately 40 min.

Flavour congeners: gas chromatographic analysis

The following free fatty acid ethyl esters (and associated analytes) were analysed by gas chromatography (GC): ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, 2-phenethyl acetate, 2-phenethyl ethanol, ethyl tetradecanoate, ethyl hexadecanoate, ethyl-9-hexadecenoate, ethyl octadecanoate, ethyl oleate (C 18:1), ethyl linoleate (C 18:2) and ethyl linolenate (C 18:3). A Hewlett Packard 5890 Series II Gas Chromatograph fitted with a Varian DB-Wax ETR column (length 60 m, ID 0.32, 1.0 μm) and a flame ionisation detector was employed. Samples (500 μL) were pre-treated with 70% (v/v) ethanol prior to direct injection (splitless mode) with 0.5 μL new make spirit. The following major volatile congeners (higher alcohols and other analytes) in new make spirit were similarly analysed by GC; acetaldehyde, ethyl acetate, acetal, methanol, iso-amyl acetate, n-butanol, n-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, isobutanol and furfural using a Hewlett Packard 6890 Series gas chromatograph, fitted with Chrompack CP Wax CB57 column (length 50 m, ID 0.25 mm, film thickness 0.2 μm) and flame ionisation detector.

Flavour congeners: sensory analysis

The sensory quality of the new make spirits, was evaluated using Quantitative Descriptive Analysis (QDA) which was used to profile their flavour characteristics[20,29]. The new make spirit samples were diluted to 20% ethanol (v/v) and 20 mL of this presented in 130 mL clear nosing glasses. Thirteen trained members of the SWRI Sensory Panel assessed the aromas of the samples, giving scores for the following 15 attributes: pungent, peaty, feinty, cereal, floral, green/grassy, fruity/estery, solventy, oily, sour, soapy, sulphury, meaty, stale and clean. These are known to be key flavour characteristics of Scotch new make spirit and the vocabulary derives from the standard flavour wheel[17,27] developed by the Scotch Whisky Research Institute, which covers all of the main flavour and aroma characteristics encountered in new make spirit and mature Scotch Whisky. A computerised data collection system was used (Compusense 5 V3.8, Compusense Inc., Guelph, Canada) with data being exported into two other software packages for further analyses; Microsoft Excel 2000 (Microsoft Corporation) and Unistat (Unistat Ltd., London).

Principal component analysis (PCA) was used to compare the sensory analysis data with a standard SWRI model for Scotch malt whisky new make spirit character.

RESULTS AND DISCUSSION

Fermentation performance of selected Brazilian yeasts in malt wort

Brazilian fuel alcohol yeasts normally encounter high concentrations of sucrose in the form of sugarcane juice or molasses in distillery fermenters operating at temperatures in excess of 30°C (see Table I). In the context of Scotch whisky production, it was therefore of interest to investigate their ability to ferment high concentrations of maltose, in the form of malt distilling wort, under standard distillery conditions and also at elevated temperatures. Our working hypothesis was that the fuel alcohol yeasts would prove to be inherently more stress tolerant compared to existing yeast strains employed in Scotch whisky distilleries.

Sugar utilisation profiles of CAT 1 and M Type yeasts

Glucose, fructose, mannose, galactose, sucrose and maltose can all be utilised by *S. cerevisiae* for growth and metabolism, with raffinose being partially utilised[32]. The ability of industrial yeast strains to ferment maltotriose is variable, but *S. cerevisiae* is unable to ferment starch. As CAT 1 was identified the best performer of the Brazilian yeasts in previous work[5], it was of interest to evaluate the capabilities of this strain to utilise different carbohydrates and to compare this with the Scotch whisky M Type yeast.

Figure 1 shows the sugar assimilation results for these two yeasts grown at 30° and 40°C in YNB medium supplemented with individual sugars (see Experimental). At 30°C M Type yeast utilised maltose and maltotriose whereas CAT 1 used maltose to a lesser extent and was incapable of using maltotriose, which perhaps explains why M Type yeast produces slightly higher alcohol yields than CAT 1 yeast (see Table II and Fig. 2), and is an important observation since the ability of yeast to ferment maltotriose is an essential requirement for Scotch whisky distilling yeast.

Interestingly, at 40°C CAT 1 exhibited better fermentation of glucose, fructose and sucrose than the M Type yeast which showed virtually no fermentation of these sugars at the elevated temperature. This supports our previous findings of a higher stress tolerance of CAT 1 when

compared with M Type yeast[6]. At all temperatures studied (25, 30, 35 and 40°C), both yeasts were unable to assimilate or ferment lactose and melibiose, but showed aerobic growth without fermentation with raffinose and galactose (data not shown). From the sugar utilisation results, it is clear that CAT 1 yeast cells are more thermotolerant than M Type yeast cells, as the ability to utilise sugars decreased for the M Type when the temperature increased to 40°C.

Relative fermentation performance and stress tolerance of CAT 1 and M Type yeasts

High gravity fermentations result in increased stresses on yeast due to osmotic pressure and ethanol. The latter deleteriously affects the cell membrane of yeast by modifying its lipid bilayer leading to leakage of cellular contents and cell death[28]. These effects are exacerbated at higher temperatures[22].

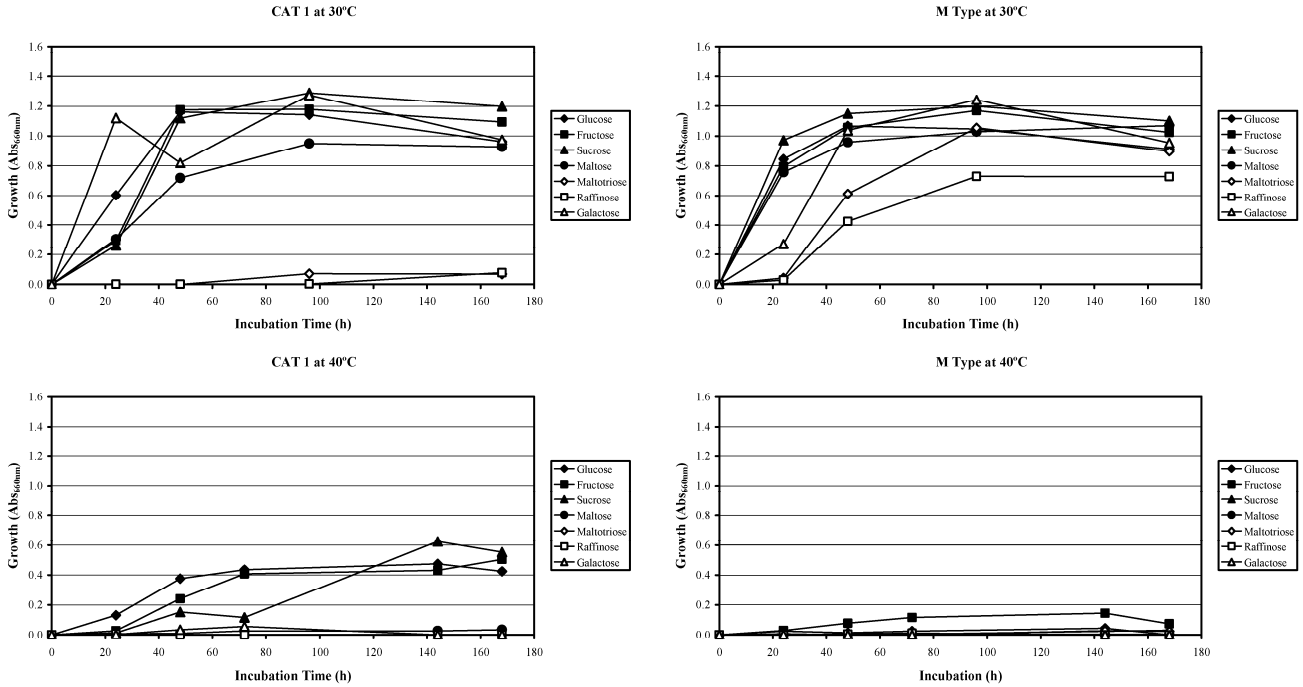

Fig. 1. Sugar utilisation profiles of CAT 1 and M Type yeast at 30°C and 40°C.

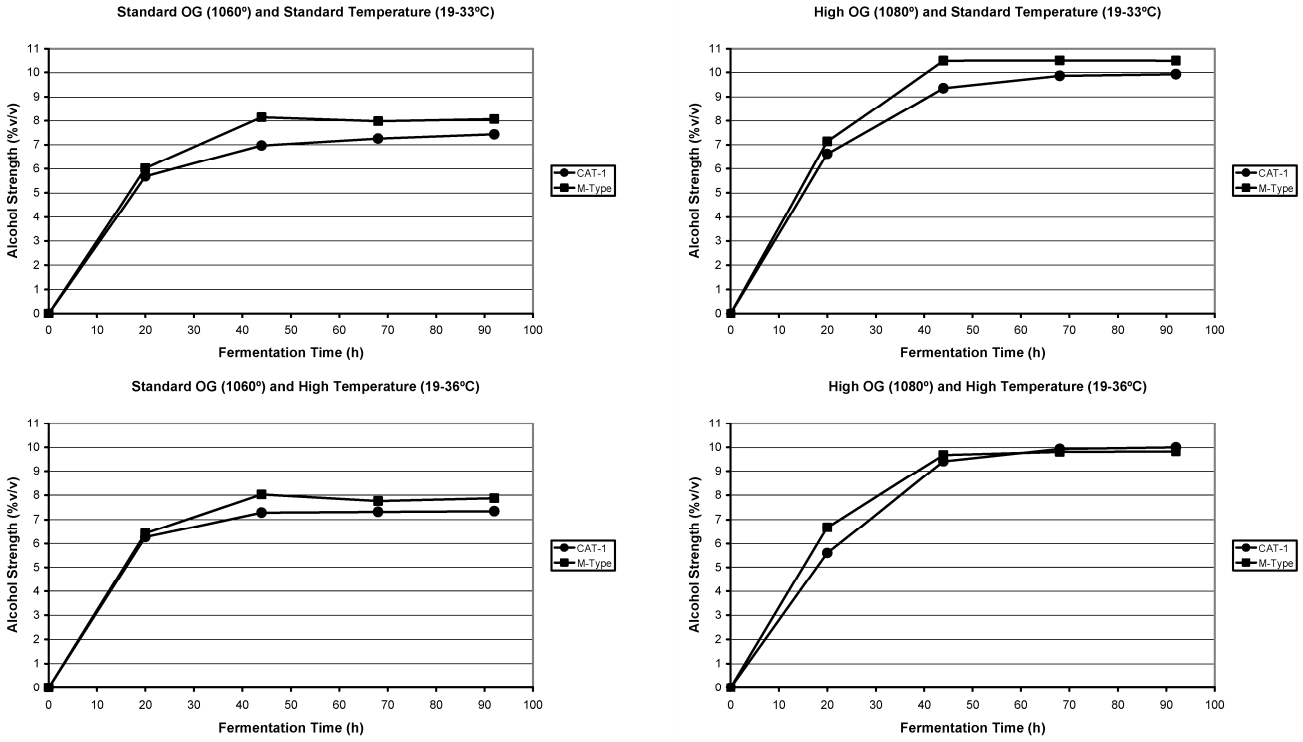

Fig. 2. Fermentation performance of CAT 1 and M Type yeast: Wash alcohol strength (% [v/v]) under different fermentation conditions.

Figure 2 shows the relative fermentation performances of CAT 1 and M Type yeasts under different fermentation conditions (OG 1060° and 1080°; 19–33°C and 19–36°C). These results indicate that although the Brazilian fuel alcohol yeast CAT 1 performs reasonably well, it doesn't quite achieve the levels of wash alcohol produced by the M Type whisky strain, since M Type has the advantage of being able to utilise certain wort sugars more effectively than CAT 1 (maltose and maltotriose as shown in Fig. 1). The only experiment where CAT 1 produces comparable levels of alcohol to M Type yeast is under conditions of increased wort gravity (1080°) and high fermentation temperature (19–36°C) which reflects an underproduction of alcohol by M Type, rather than an enhanced production of alcohol by CAT 1. The M Type yeast may have the advantage of being able to utilise certain malt wort substrates to produce more alcohol, but CAT 1 has the advantage of being more stress tolerant under adverse fermentation conditions and maintains a relatively high viability during all of the fermentation experiments studied, e.g., 50% cf. 2% viability after 3 days, as shown in Fig. 3 (19–36°C and 1080°).

It is apparent from Fig. 3 that CAT 1 is better adapted to tolerate elevated temperatures compared with the M Type. This strain presumably exhibits an adaptive stress response[32] resulting from exposure to elevated temperatures, enabling yeast metabolic activity to continue under the rigours of a typical Scotch whisky fermentation. This

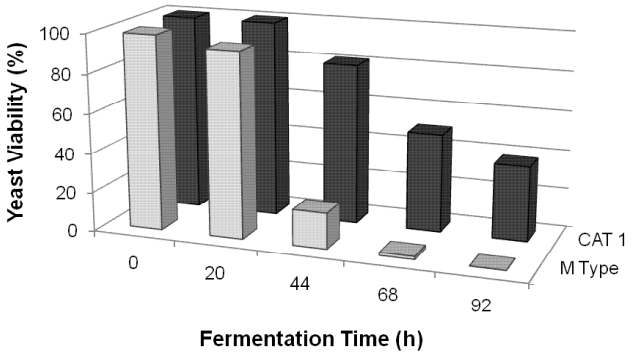

Fig. 3. Percentage viability of CAT 1 and M Type yeast at high gravity (1080°) and high temperature (19–36°C).

enhanced thermo-tolerance may be due to elevated intracellular levels of trehalose[33] and glycerol, and/or increased synthesis of heat shock proteins[10,33]. Heat shock proteins perform molecular 'chaperoning' functions in the yeast cells, assisting in the degradation of stress damaged proteins. This is achieved by enhancing the flow of substrates through proteolytic pathways[32,33].

The overall stress tolerance characteristics of CAT 1 may prove of benefit if high-viability distiller's yeast was to be re-pitched for subsequent fermentations. Although this is not a current practice in Scotch whisky distilleries, it is commonly employed in Brazilian fuel alcohol plants. Nevertheless, in the former situation, the ability to predict and control yeast viability and vitality is essential to ensure optimum fermentation performance and to guarantee a uniform and stable product.

Impact on flavour/aroma congeners

Many yeast fermentation metabolites contribute in important ways to the flavour of distilled beverages[9,17]. The most important components influencing the quality of whisky new make spirits are volatile congeners such as higher alcohols and volatile esters. Esters are particularly important in determining the sweet and fruity characteristics of fermented beverages. These are formed as a result of the intracellular condensation of acyl CoA esters of fatty acids with alcohols[14]. Although of lesser importance, fatty acids can also have important flavour effects in beverages.

In general, both yeast strains and wort gravity had significant effects on the levels of congeners, with CAT 1 on the whole producing larger amounts of the higher alcohols (isobutanol, 2-phenylethanol) (Table III) and smaller amounts of esters than the M type yeast (Table IV). Only in the case of the lower temperature profile (19–33°C) fermentation, did CAT 1 give higher levels of esters. However, at the higher temperature (19–36°C) the magnitudes of the differences between the yeasts were lower, and at the higher temperature there was little difference in the amount of short chain esters generated by the yeasts.

As has been observed for high gravity brewing[28], the spirits produced using high gravity wort had significantly higher levels of esters (Table IV) than the standard gravity

Table III. Levels of major volatile congeners in new make spirit (results expressed as g/100 L abs. alc.).

	Standard temperature profile (19–33°C)				High temperature profile (19–36°C)			
Congener	**M Type 1060°**	**CAT 1 1060°**	**M Type 1080°**	**CAT 1 1080°**	**M Type 1060°**	**CAT 1 1060°**	**M Type 1080°**	**CAT 1 1080°**
Acetaldehyde	2.3	2.7	4.2	3.6	9.8	4.5	5.0	4.7
Ethyl acetate	50.0	38.9	42.2	42.4	39.3	29.1	39.8	35.9
Acetal	7.9	3.1	7.4	3.1	5.2	6.3	5.3	[0.6]
Methanol	4.8	5.8	4.7	5.1	5.1	5.6	5.1	5.0
n-Propanol	32.8	24.8	26.8	22.5	31.2	23.1	27.0	20.4
iso-Butanol	42.8	47.4	29.4	41.9	43.1	46.1	30.8	42.3
iso-Amyl acetate	2.7	3.3	1.6	3.0	1.5	1.6	1.2	1.5
n-Butanol	[0.3][a]	<LOD[b]	[0.5]	[0.2]	[0.4]	<LOD	[0.3]	[0.2]
Total iso-amyl alcohols	150.7	191.1	124.0	162.5	152.0	178.4	124.0	154.6
2-Methyl-1-butanol	38.7	35.1	29.3	30.9	38.3	32.9	28.7	29.6
3-Methyl-1-butanol	112.0	156.0	94.8	131.6	113.7	145.5	95.2	125.0
Total higher alcohols	226.3	263.2	180.3	226.8	226.3	247.5	181.7	217.3
Furfural	1.3	3.5	0.7	0.8	1.4	2.9	0.7	0.8

[a] Brackets indicate a value outside calibration range.
[b] Below level of detection.

Table IV. Levels of ethyl esters and fatty acids in new make spirit (results mg/L at sample strength).

	Standard temperature profile (19–33°C)				High temperature profile (19–36°C)			
Congener	**M Type 1060°**	**CAT 1 1060°**	**M Type 1080°**	**CAT 1 1080°**	**M Type 1060°**	**CAT 1 1060°**	**M Type 1080°**	**CAT 1 1080°**
Ethyl hexanoate	5.0	4.6	8.2	6.2	3.7	2.7	4.2	4.3
Ethyl octanoate	26.6	18.0	41.2	24.2	21.0	13.2	22.8	18.5
Ethyl decanoate	[115.3][a]	59.4	[138.8]	81.5	80.4	53.8	89.7	61.4
Ethyl dodecanoate	98.3	15.8	[109.5]	26.6	68.6	26.4	93.6	29.1
Ethyl tetradecanoate	7.0	[1.2]	12.9	2.8	6.0	2.3	12.4	3.5
Ethyl hexadecanoate	11.4	2.6	15.6	6.3	7.1	5.4	21.3	11.9
Ethyl 9-hexadecenoate	14.3	4.1	13.5	4.2	10.3	6.6	19.2	6.2
Ethyl C18:0 (stearate)	<LOD[b]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	< LOD
Ethyl C18:1 (oleate)	[0.8]	<LOD	<LOD	<LOD	<LOD	<LOD	[1.2]	[1.4]
Ethyl C18:2 (linoleate)	[0.8]	<LOD	<LOD	<LOD	<LOD	<LOD	[1.0]	< LOD
Ethyl C18:3 (linolenate)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	< LOD
Hexanoic acid	3.8	4.9	<LOD	<LOD	<LOD	<LOD	<LOD	< LOD
Octanoic acid	28.0	37.8	5.9	6.1	19.9	31.4	5.8	5.0
Decanoic acid	50.3	76.4	6.5	7.8	32.3	59.8	7.6	6.9
Dodecanoic acid	24.7	20.2	2.2	2.9	14.6	19.4	3.8	2.8
2-Phenethyl acetate	5.8	9.0	[1.5]	2.0	4.2	6.9	[1.0]	[1.2]
2-Phenethyl ethanol	21.9	35.4	2.7	4.1	21.5	25.5	5.2	4.5

[a] Brackets indicate a value outside calibration range.
[b] Below level of detection.

Table V. Flavours associated with the major volatile congeners found in new make spirit.

Congeners	**Flavour**
Acetaldehyde	Pungent, ethereal
Acetal	Fruity, tart
Ethyl acetate	Pineapple, ethereal, solventy
Iso-amyl acetate	Fruity, bananas, pears
n-Propanol	Alcohol, sweet
Iso-butanol	Alcohol, wine-like
n-Butanol	Medicinal
Methanol	Alcohol, pungent, slightly fruity
2-Methyl-1-butanol	Fusel oil
3-Methyl-1-butanol	Fusel oil
Furfural	Sweet, nutty, cereal

spirits, and the concentrations of medium-chain esters (from ethyl hexanoate to ethyl hexadecanoate) were all increased at high OG.

It is interesting to note that comparison of congener analysis data with sensory assessments indicates that the higher levels of esters present in the M Type spirits do not necessarily influence the perceived 'fruity/estery' character of the spirit. This indicates that actual levels of esters present in the spirit are not the only factors determining its 'estery' character and that sensory analysis of sample aroma characteristics give a better indication of spirit quality.

Sensory analysis

Flavour is extremely important in the production of Scotch whisky and it is essential that any new make spirit produced conforms to acceptable sensory profiles typical of Scotch whisky new make spirit. Therefore, spirit produced using new yeast strains under different fermentation conditions must have recognisable flavour characteristics typical of new make spirit, to be considered suitable for further investigation. Many flavour active compounds are present in new make spirit which contribute to its overall sensory profile. Typical aromas associated with some of the major volatile components found in new make spirit are highlighted in Table V.

A team of trained sensory analysts carried out Quantitative Descriptive Analysis (QDA) on the new make laboratory spirits produced using CAT 1 and M Type yeast under different fermentation conditions. A range of standard descriptors were used to express the sensory profile of the distillates such as peaty, green/grassy, fruity/estery, sweet, solventy, sulfury and stale. Sensory results are recorded as spider diagrams in Fig. 4. These diagrams show that despite relatively large quantitative differences in congener levels observed in CAT 1 and M Type new make spirits, sensory profiles were similar with only subtle differences being observed under certain fermentation conditions, e.g., CAT 1 yeast produced a heavier spirit with more feinty, cereal, oily, sour and soapy notes than M Type yeast under standard fermentation conditions (1060° and 19–33°C).

Spirit flavour is known to be extremely complex and direct relationships between concentrations of flavour congeners and their perceived sensory character are not necessarily observed[17]. It is considered that the concentration and balance of congeners in the spirit can affect its overall sensory profile with certain congeners interacting to either enhance or mask aromas. Also, some congeners such as long chain fatty acid ethyl esters can interact with volatile flavour active congeners trapping them in the liquid phase of the spirit thus preventing them from contributing to the overall aroma[18].

To aid in the interpretation of the sensory data represented in the spider diagrams, Principal Components Analysis (PCA) was used and the distribution of new make spirits across principal components 1 and 2 was examined to determine whether any separation according to yeast strain, wort gravity or fermentation temperature could be observed (Fig. 5). CAT 1 new make spirit showed some separation due to the effects of wort gravity whereas spirits produced by M Type yeast were more influenced by fermentation temperature.

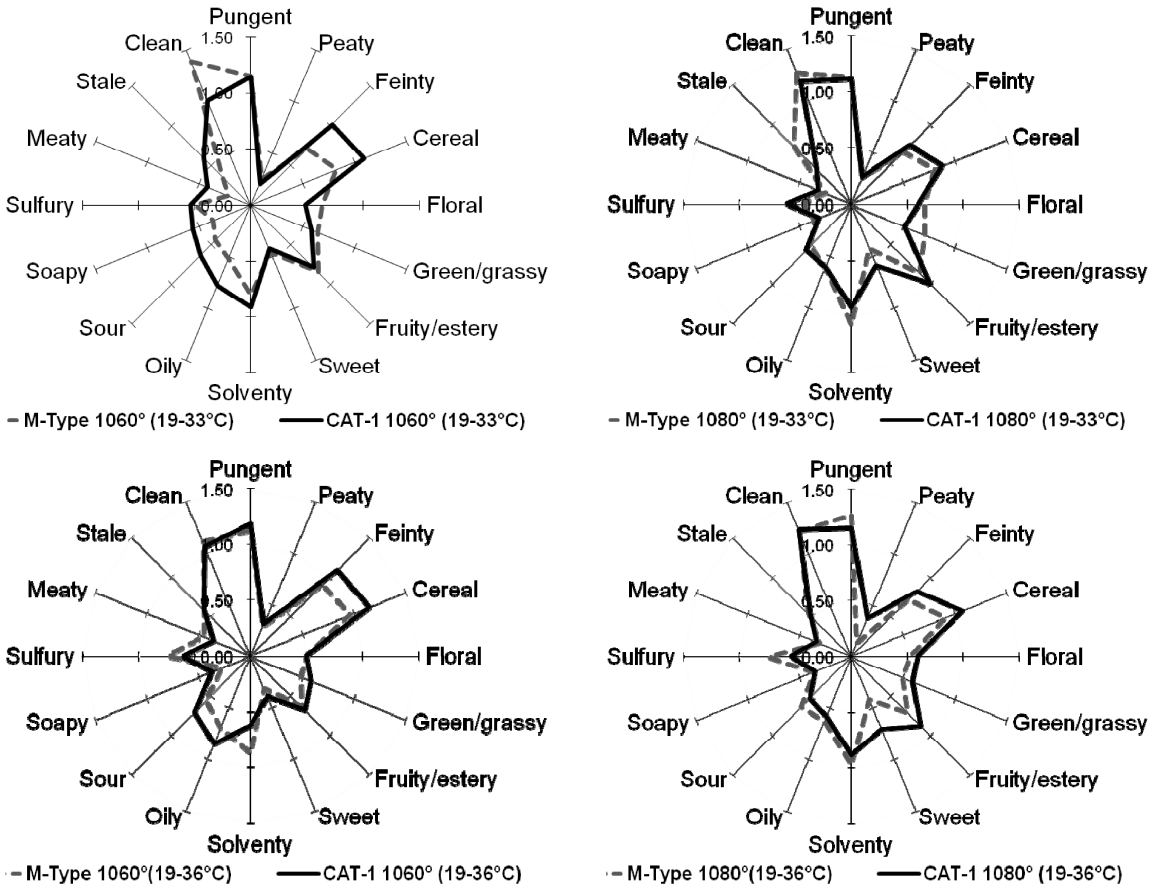

Fig. 4. Flavour profiles of CAT 1 and M Type new make spirit at standard (1060°) and high (1080°) wort gravity and standard (19–33°C) and high fermentation temperature (19–36°C).

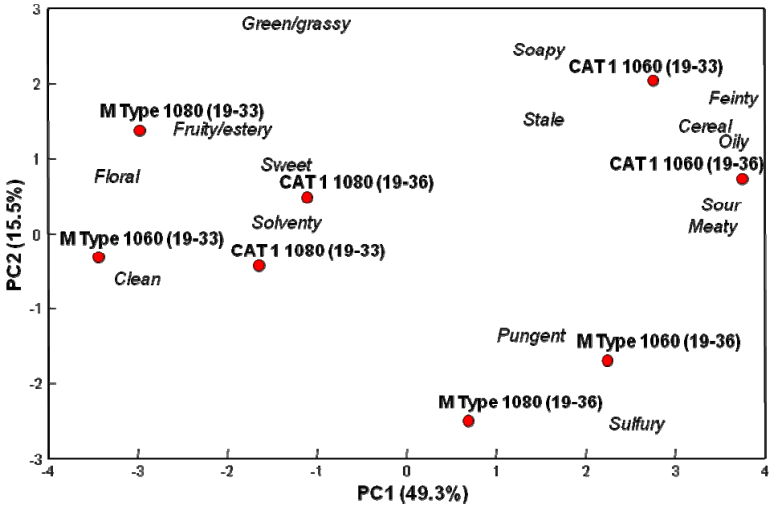

Fig. 5. PCA plot of new make spirit sensory data.

However, in general, the results confirm that the flavour characteristics of the spirits produced by CAT 1 and M Type yeast under different fermentation conditions were consistent with what would be regarded as 'typical' Scotch whisky new make spirit.

CONCLUSIONS

In some respects, it was rather surprising to us to find that Brazilian fuel alcohol yeast strains compared favourably with a commercial whisky distilling yeast in trial fer-

mentations. One interesting aspect of strain CAT 1 was that it maintained high levels of viability during the late/final stages of fermentation. This attribute, which is important for fuel ethanol production, may also be significant for Scotch whisky processes in terms of flavour[36] and for potential recycling of distilling yeasts as it has been shown that yeast cell death at the end of fermentation directly contributes to spirit consistency and quality.

This study has highlighted the potential application of novel yeast strains for Scotch whisky fermentations, and confirmed that CAT 1 not only showed reasonable fermentation performance, but also produced spirit of acceptable quality. However, while CAT 1 may have the potential to provide some benefits, particularly with regard to maintenance of high culture viabilities at the end of fermentation, the inability of CAT 1 to utilise maltotriose is a clear disadvantage in fermenting Scotch whisky type worts. One reason for this is that the Brazilian yeast strains that have been studied were isolated from environments where the predominant sugar available is sucrose, rather than maltose, which is the norm for cereal (starch) based spirits converted with malt enzymes, such as Scotch whisky. If this problem could be overcome, CAT 1 or a similar yeast strain would have potential as a new yeast strain for the Scotch whisky distilling industry, particularly if higher wort gravities and higher temperatures are to be employed.

The study highlights the potential of exploring the considerable biodiversity of yeasts, to identify stress tolerant strains that are capable of efficiently utilising maltotriose, which would be useful for the production of cereal based distilled spirits.

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