Influence of Zinc on Distiller's Yeast: Cellular Accumulation of Zinc and Impact on Spirit Congeners

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ABSTRACT

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Accumulation of zinc by a whisky distilling yeast strain of *Saccharomyces cerevisiae* was studied during fermentation of malt wort and synthetic defined medium. Zinc uptake by yeast cells was very rapid in malt wort, as zinc (0.32 µg/mL) was completely removed from the fermentation medium within one hour. The type of fermentable carbohydrate had an impact on the kinetics of zinc accumulation, with maltose most effective at enhancing metal uptake at zinc concentrations above 3.2 µg/mL. Enriching yeast cells with zinc by "preconditioning" impacted on the production of flavour congeners in the distillates produced from fermented cultures. Such distillates were characterised by an altered flavour and aroma profile. In particular, the production of some higher alcohols increased when yeast cells were preconditioned with zinc. This phenomenon is yeast strain related. Industrial fermentation processes, including brewing and distilling, may benefit from optimization of zinc bioavailability in yeast cultures resulting in more efficient fermentations and improved product quality.

Key words: metal ions, micronutrients, *Saccharomyces cerevisiae*, whisky distilling, zinc.

INTRODUCTION

Zinc is a trace element of primary importance for yeast growth and metabolism. This metal is used as cofactor in numerous enzymes[32] and plays a structural and functional role in proteins and nucleic acids[1,26,31]. For yeast fermentation processes, zinc is absolutely essential for alcohol production, as it is required for the functioning of the terminal enzymatic step in ethanologenesis, namely alcohol dehydrogenase[20]. In some industrial yeast-based processes, such as wine-making, zinc concentrations in the medium (e.g. grape must) are normally deemed satisfactory and it is unusual to carry out zinc analyses and zinc supplementations[9]. However, other industrial fermentation processes may require zinc supplementations to ensure optimal zinc bioavailability and this is the case in brewing, where zinc concentrations are occasionally below minimum levels for satisfactory fermentation performance[7].

Yeast cells accumulate zinc biphasically[24]: the first phase consisting of a metabolism-independent zinc binding to sulphydryl residues within the cysteine groups of the cell wall[3], and the second phase characterised by active transport of zinc inside the cell. Zinc is then subsequently translocated to the yeast vacuole[8,18,19,22]. Temperature, pH and metabolic inhibitors all influence zinc sequestration by yeast cells[11,12,24,28,35].

In the brewing process, malt wort boiling may reduce zinc bioavailability as the metal may form complexes and precipitates with proteins, such as cysteine groups of peptides and amino acids[14]. Zinc required for yeast growth and metabolism may therefore be compromised leading to slow and incomplete fermentations. Several studies have described zinc cell accumulation by yeast and have defined optimal zinc concentrations in brewing fermentations[3,4,8,29]. The consensus of such studies is that, although zinc interactions are yeast strain-dependent, concentrations around 0.25–0.50 µg/mL appear to be optimal for cell growth, and 1–2 µg/mL for glycolysis[16]. Generally speaking, when zinc concentrations fall below around 0.1 µg/mL, fermentations may become sluggish[4,13,15].

Very few brewing and distilling studies have been conducted with regard to the effects of zinc on production of flavour congeners, such as higher alcohols and other volatiles, by yeast. Skanks et al.[29] have shown that zinc additions increased the levels of higher alcohols and esters but reduced acetaldehyde levels. Although addition of 0.5 µg/mL zinc increased volatile organic compounds, this may also increase the concentration of medium chain fatty acids (MCFA) responsible for undesired soapy, fatty and rancid tastes[33]. Similarly, few studies have described zinc cell uptake by industrial yeast strains[8,9] and the optimisation of zinc levels in wort during fermentation[23].

The present study describes zinc accumulation from malt wort by a whisky distilling strain of the yeast *Saccharomyces cerevisiae* and the impact of various sugars on zinc uptake. The effect of yeast zinc-preconditioning was also investigated with regard to impact on spirit congeners such as higher alcohols and esters. The work has industrial implications for the production of distilled beverages, since zinc influences both yeast fermentation performance and product quality.

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MATERIALS AND METHODS

Yeast strains and growth media

Experiments were carried out with a distilling yeast strain of *S. cerevisiae* "M-type" (from Kerry Biosciences, Menstrie, UK). Experiments related to the effect of zinc preconditioned yeast cell cultures on distillation were executed with both M-type and an ale brewing yeast strain from the National Collection of Yeast Cultures (N.C.Y.C. – UK http://www.ncyc.co.uk/ No. 1681).

Studies on the effect of carbohydrates on zinc accumulation were carried out using Yeast Propagation Medium (YPM), a modified version of Edinburgh Minimal Medium (EMM3)[21]. Glucose, fructose, maltose or sucrose were supplied (at 30 g/L) as carbon sources in YPM which also contained: ammonium sulphate 5 g/L, ammonium dihydrogen phosphate 2.84 g/L, potassium chloride 2 g/L, magnesium sulphate heptahydrate 1 g/L, calcium chloride dehydrate 30 mg/L, potassium iodide 0.15 mg/L, manganese sulphate 0.6 mg/L, copper sulphate 60 µg/L, citric acid 1.5 mg/L, molybdic acid 0.24 mg/L, ferric chloride 0.3 mg/L, boric acid 0.75 mg/L, nicotinic acid 40 mg/L, inositol 40 mg/L, calcium pantothenate 4 mg/L, thiamine-HCl 1.6 mg/L, pyridoxine-HCl 1.6 mg/L and biotin 40 µg/L. The pH was adjusted to 4.5. Zinc concentrations of YPM medium were adjusted at 0, 0.8, 1.6, 3.2 and 6.4 µg/mL, using a 4,000 µg/mL sterile stock solution of zinc sulphate heptahydrate (Fluka Chemie AG, Switzerland).

Malt wort was prepared by infusion mashing using a 25% (w/v) suspension of barley malt grist (Optique variety) in pre-heated (65°C) deionised water. The mash was kept at 65°C for 1 h. Final malt wort was filtered, autoclaved at 120°C for 20 min and clarified by centrifugation in order to separate precipitated proteins. Analyses of malt worts were carried out to determine original gravity (1062 OG), pH and zinc levels. Zinc levels were adjusted by calculated aliquots of a zinc acetate stock solution. The use of zinc acetate or zinc sulphate was previously found not to influence zinc accumulation by yeast cells[5].

Maintenance of zinc-free conditions

All glassware and flasks used in the described experiments were deionised according to the following procedure: soaked in 2% nitric acid for 12 h, washed with deionised and distilled water (ddH_2O), rinsed with 0.1 M EDTA and four times washed with ddH_2O prior to final drying. This procedure was essential to remove any contaminating ions, including zinc[6].

Growth conditions

Yeast seed culture inocula were prepared from refrigerated slope cultures by inoculating into liquid media (250 mL), in 500 mL Erlenmeyer shake flasks (in an orbital shaker at 25°C, 200 rpm) of a loopful of cells. After 24 h of growth, cells were counted using a bright field microscope and haemocytometer and a calculated amount of seed culture volume was centrifuged, cells were washed once with sterile deionised water and resuspended in experimental media to give an initial cell number of 5×10^6 cells/mL. Malt wort at 0.32 µg/mL zinc was used in the first experiments (zinc accumulation by distilling yeast in malt wort) and YPM (0.8 µg/mL Zn) was the seed medium used for experiments in the second section (influence of carbohydrate sources on zinc accumulation by distilling yeast). In experiments described in the third section (effects of Zn-preconditioned yeast), cells were Zn-preconditioned in malt wort supplemented with 0.5 µg/mL zinc acetate for 48 h. Control cultures were grown in the same medium without zinc supplementation. Washed cells (in deionised water) were then inoculated into shake flasks at initial cell densities of 5×10^6 cells/mL in an orbital incubator at 200 rpm and 25°C.

A haemocytometer (Neubauer Improved type) was employed to count yeast cell numbers using a bright field microscope. Methylene violet was used to assess yeast cell viability according to the method of Smart et al.[30] Mean yeast cell volumes were measured using a Coulter Counter Multisizer (Beckman-Coulter Electronics, Luton, UK).

Distillation

Once the fermentations were complete, the fermented 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. The low wines were redistilled and three distillate fractions were collected: firstly, the *foreshots*; secondly, the *new make spirit* (~150 mL, retained for congener analyses); and finally, the *feints*. Distillations were conducted at the Scotch Whisky Research Institute (Edinburgh, UK).

Metal ion analyses

Yeast cells were washed thrice in deionised water and hydrolysed with concentrated nitric acid (69% Analar grade from Fisher Scientific, Loughborough, UK), at 90°C for 1 h. This completely solubilised the yeast cells producing a clear solution for zinc cell content analysis. Malt wort supernatants were obtained after centrifugation and treated with nitric acid (1:1) at room temperature in order to hydrolyse possible metal-chelating proteins. Hydrolysates of yeast and medium were diluted and zinc concentrations were analysed using a Perkin Elmer 1100B atomic absorption spectrophotometer. Each sample was analysed in triplicate.

Ethanol analyses

Ethanol production during fermentation was analysed using a Gas Chromatograph Mass Spectrometer GCMS-QP2010 (Shimadzu, Japan) fitted with an Agilent HP Blood Alcohol capillary column (ID: 0.32 mm, length: 7.5 m, film: 20 µm). Program conditions were as follows: column temperature 125°C, injector temp 250°C, split ratio 20:1, linear velocity 200 cm/sec, detector temperature 250°C, temperature program: 125°C, rate 15°C/min, final temperature 150°C. Ethanol in distillates was measured using an Anton Paar DMA 500 density meter calibrated against air (density of 0 g/mL^3) and boiled deionised water (density of 0.99715 g/cm^3). Readings were taken at 20°C after each sample was previously flushed thrice in the instrument.

Analyses of higher alcohols and esters in distillates

A gas chromatograph (Hewlett Packard 6890) was used for the determination of higher alcohols in distillates (new make spirit). Separation was carried out in a 50 m capillary column packed with CP wax CB57 using a pulsed injection system. Ethanol at 40% (v/v) was used as the standard and n-pentanol as the internal standard. Carrier gas was H_2 at a flow rate of 3 mL/min, split 1 µL injection, split ratio 25:1, split flow rate 150 mL/min, oven program 35°C ramped to 120°C at 7°C /min, detector temperature: 250°C.

For determination of esters, a gas chromatograph (Hewlett Packard 5890 series II) was used and fitted with an autosampler (Hewlett Packard 7673A) and flame ionisation detector. The analytical column was packed with Quadrex. Gas carrier was H_2, oxidant air 300 mL/min, split flow rate 40 mL/min, injector mode splitless, injector volume 0.5 µL, injector temperature 230°C, detector temperature: 260°C.

Statistical analyses

For all experimental conditions, either duplicate or triplicate sample analyses were carried out. Students t test was applied to compare data of different experimental conditions and to determine statistical significance. Error bars in graphs denote standard deviations.

RESULTS AND DISCUSSION

Zinc accumulation by distilling yeast in malt wort

Cells of the distilling yeast, *S. cerevisiae* strain M-type, were grown in malt wort and evaluated for growth and zinc cell accumulation during the first 7 h of fermentation (Fig. 1) and after 24 h (data not shown). Zinc was removed from the medium after only 30 min by the inoculum of yeast cells, which concomitantly reached their highest zinc cell content (47 fg/cell) after 40 min, a value around 5 times higher compared to the initial zinc cell content (8.9 fg/cell). Cell viabilities were not affected in the period of time under evaluation (99% viable). In this period, yeast cells started to divide and cell number increased. Contrary to industrial fermentations of malt worts (e.g. in breweries and whisky distilleries) that are mostly static or with a mild agitation, the model system used in our experiments was based on vigorous agitation. Such a system may contribute to the fast zinc cell uptake. De Nicola and Walker[8] have recently reported similar zinc uptake patterns in a lager brewing strain in malt wort, as well as in a wine yeast strain fermenting grape must[9]. It appears that this rapid zinc uptake was not strain or medium dependent; however, maximum zinc accumulation capacity by yeast cells appeared related to cellular volumes. Mean cell volume (MCV) of the distiller's yeast strain in this study was on average 125 fL. In comparison with the distiller's yeast strain employed, in the same growth conditions, zinc cell content in a lager beer yeast strain was found to peak higher (84 fg/cell) at MCV 186 fL, and lower (30 fg/cell) in a wine strain at MCV 52 fL[5]. Zinc intracellular concentration was 0.376 g/L in the distiller's yeast, 0.452 g/L in the lager strain and 0.577 g/L in the wine yeast. Interestingly, the wine yeast accumulated the lowest zinc content but achieved the highest zinc concentration. The difference in zinc concentration did not depend on cell competition for zinc as the final cell number was similar in the wine and distiller's strain population (10^8 cells/mL). Since zinc translocates primarily to the yeast vacuole[8,18,19,22], it is conceivable that yeast with

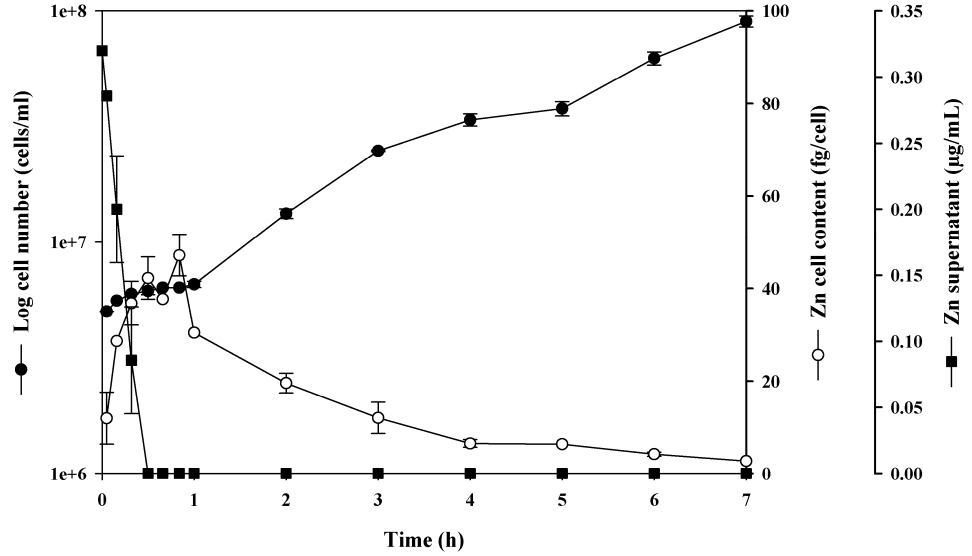

Fig. 1. Zinc accumulation by a distilling yeast strain in malt wort. The distilling yeast strain *S. cerevisiae* M-type was cultivated in shake flasks, in malt wort (zinc at 0.32 µg/mL), at 25°C, 200 rpm and for 24 h. Figure represents cell growth, zinc cell content and zinc supernatant concentrations in the first 7 h of growth. Zinc cell content was expressed on a per cell basis after cell hydrolysis and analysis by atomic absorption spectrophotometer (AAS). Error bars denote standard deviations. Statistical significance by t test: $p<0.05$.

larger cell volumes, and consequently bigger vacuoles, are able to accumulate more zinc than smaller cells. However, the capability to accumulate zinc at higher concentrations could also depend on other factors such as the presence of Zn-binding intra-vacuolar components (e.g., polyphosphate bodies)[17].

During the progress of the fermentation, yeast cell growth reached numbers close to 10^8 cells/mL and when we express zinc content per cell, the mean cellular zinc content at the end of fermentation in individual yeast cells was greatly reduced compared to cells at the onset of fermentation.

Zinc uptake into cells is mediated by plasma membrane ATPase activity via a transmembrane proton gradient[25]. Energy required for metal transport by yeast may rely firstly on sufficient intracellular carbohydrate reserves (e.g., glycogen) previously accumulated during inoculum culture growth and subsequently to the carbohydrates (primarily maltose) present in malt wort. Figure 2 shows the influence of wort sugars on zinc accumulation. Zinc supplementations to yeast cultures appear effective when applied during the early stages of the fermentation, when the availability of energy sources is greatest. Other parameters such as temperature[8,24] and pH[11,12] have previously been observed to influence zinc uptake in yeast cells, presumably by modulating membrane ATPase activity.

Influence of carbohydrate sources on zinc accumulation by distilling yeast

Zinc uptake was studied during the initial 6 h of growth in YPM medium containing maltose, glucose, fructose or sucrose supplied at concentrations of 30 g/L. Zinc concentrations were adjusted in the range 0–6.4 µg/mL. These concentrations are representative of malt worts used in the brewing process[27]. The synthetic medium YPM enabled us to determine zinc accumulation by yeast cells regardless of any chelating effect of complex compounds present in malt wort (e.g. polyphenols, proteins). All zinc in YPM was assumed to be bioavailable. In addition, zinc in the medium was solely supplied by zinc sulphate and all other medium ingredients were of high chemical purity.

Figure 2a shows clear variations in residual zinc concentrations in fermentation supernatants, denoting different effects of carbohydrate sources on zinc uptake. When glucose was used as a carbon source, zinc removal from the medium was slow in comparison with fructose (at 0.8 and 3.2 µg/mL of initial zinc concentration) and maltose (at 3.2 and 6.4 µg/mL of initial zinc concentration). Yeast cellular zinc content was very low in cells grown in fructose. Mean zinc cell content (Fig. 2b) was higher in yeast cells grown in glucose medium up to 3.2 µg/mL of initial zinc concentration. At 6.4 µg/mL initial zinc, yeast cells grown in maltose led to enhanced zinc cell content. These findings relate to differential yeast cell growth rates (Fig. 2c), as cells in fructose grew much faster in the first 6 h compared with other carbon sources (Fig. 2c). When glucose was supplied, cell growth was poor and this impacted on the high residual concentration of zinc in the medium. Cell viabilities remained at 98% at any zinc concentration studied (data not shown).

Zinc accumulation by yeast cells consists of a metabolic-independent phase at the cell wall level and an active transport phase at the plasma membrane level[3,24]. Therefore, rapid zinc accumulation by yeast is coupled with energy-yielding metabolism. Sucrose and maltose are both disaccharides. The former needs to be hydrolysed by the periplasmic enzyme invertase (α-glucosidase) to its constituent glucose and fructose before these monosaccharides are taken up by the cells. Maltose is actively taken up by yeast cells due to a plasma membrane H^+-ATPase generating a transmembrane electrochemical proton gradient[17]. Maltose is subsequently hydrolysed by the enzyme maltase into two molecules of glucose. The process of uptake and utilization is relatively slow for both

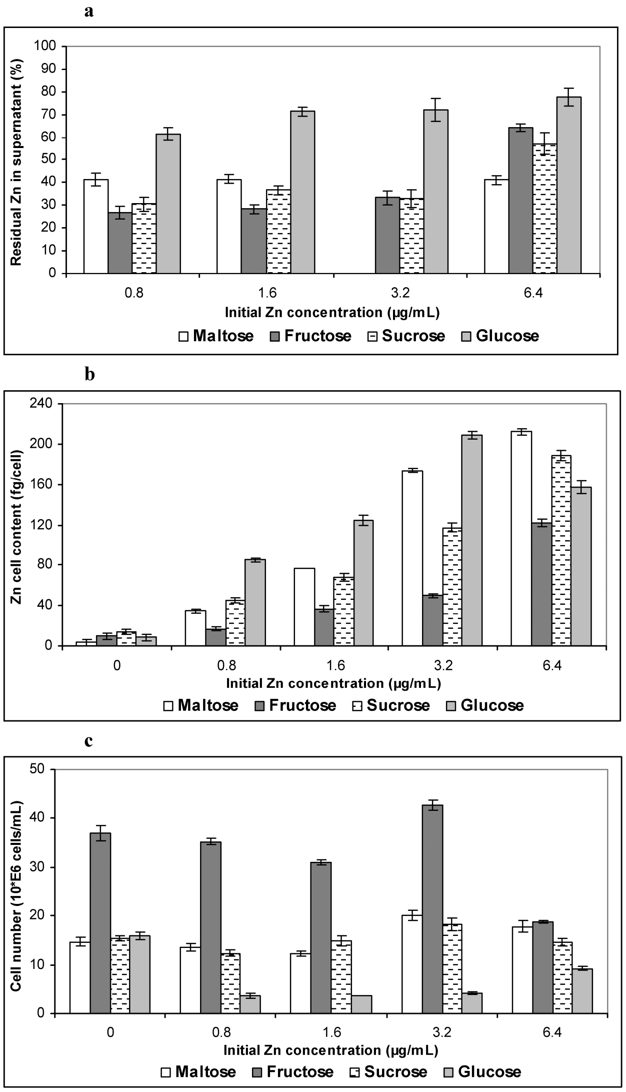

Fig. 2. Zinc accumulation and growth of a distilling yeast strain grown in YPM with variable zinc and carbohydrate sources. Residual zinc levels in media were calculated as percentages (a) considering the initial zinc levels in media and the zinc accumulated by yeast cells (*S. cerevisiae* M-type) after 6 h of growth in YPM, in shake flasks, at 25°C for 24 h. Mean zinc cellular content (b) was evaluated to assess the effect of various carbon sources on zinc accumulation. Cell growth (c) was determined by haemocytometer. Error bars denote standard deviation. Statistical significance by t test: $p<0.05$.

sucrose and maltose, in comparison with glucose. However, at zinc concentrations above 3.2 µg/mL, energy generated by maltose appeared even more effective compared to glucose and fructose with regard to rapidity of zinc accumulation.

Effects of Zn-preconditioned yeast

Zinc cell content impacted on fermentation performance (Table I). Non pre-conditioned ale brewing yeast and distiller's yeast produced higher ethanol levels compared to their Zn-preconditioned counterparts. Yeast cellular zinc content therefore appears to directly influence ethanol production. Despite the fact that alcohol dehydrogenase ADH (the terminal enzyme in ethanol fermentation) is a zinc metalloenzyme[20], yeast cells with high zinc content produced low concentrations of ethanol. This could be related to zinc accumulation in vacuoles[18,19,22,8] not made available to ADH or to the presence in the studied strains of other zinc enzymes with higher affinities for Zn.

Concerning the influence of zinc on flavour and aroma congeners in fermentation distillates, the production of total higher alcohols (Table II) was higher in the distiller's strain compared to the ale brewing strain. Zn-preconditioned cells of the brewing yeast produced higher total alcohols (+6%, $p<0.05$) compared to control (un-preconditioned) cells. No significant difference was found with the distiller's strain. However, in both strains, the concentration of various higher alcohols differed when yeast cells were preconditioned with excess zinc. Higher alcohols impart a warming character as well as an intensification of the alcoholic taste in fermented and distilled beverages. Higher alcohols are formed during the primary fermentation by yeast, either from sugars or from amino acids (Ehrlich pathway) during amino acid biosynthesis and catabolism. Amino acid concentrations in the mash and the rate at which the amino acids are taken up by the cell can influence the rate of production of higher alcohols[34].

Table II shows that methanol detected in distillates was generally very low, but using Zn-preconditioned distiller's yeast resulted in more methanol than the corresponding control. The n-butanol production in all distillates tested was also very low, but higher in the distillates from un-preconditioned yeast (this difference was 30% higher in the ale brewing yeast strain compared to the corresponding Zn-preconditioned yeast). Conversely, the concentrations of 2-methyl-1-butanol (which originates from L-isoleucine catabolism) and 3-methyl-1-butanol (originating from leucine catabolism) were higher in the Zn-preconditioned distillates. The latter of these higher alcohols was produced at the highest concentration in all distillates analysed, suggesting that leucine in the mash was present at high concentrations compared to other amino acids used for higher alcohol production. In the ale brewing yeast strain, the concentration of isobutanol was also higher in the Zn-preconditioned distillates compared to the controls and was only slightly lower in the distiller's strain than the control distillate. Isobutanol originates from metabolism of the amino acid valine. The synthesis of the branched-chain amino acids leucine, valine and isoleucine is regulated by the expression of the genes *ILV2*, *ILV3* and *BAT2*. These genes were found down-regulated under zinc-limited yeast growth in the presence or absence of oxygen[6]. Transcriptional regulation of these three genes appears to be mediated by zinc and therefore maintaining zinc concentrations at appropriate levels during fermentation may provide a suitable balance of higher alcohols.

Comparing the yeast strains of this study, the concentration of most of the higher alcohols produced by ale and distiller's strains was very similar, independent of any Zn-preconditioning. Only n-propanol (formed from the amino acid L-threonine) was found at higher levels in the distiller's strain compared to the ale strain. With regard to esters, in the ale and distiller's yeast strains, the ester concentration was higher in control distillates compared to Zn-preconditioned cells (Table III). Among the secon-

Table I. Ethanol concentration in distillates obtained from fermentations by ale and distiller yeast strains with variable Zn cellular content.

	Ethanol (%)	Zn cell content (fg/cell)
Control		
Distiller	18.20	5
Ale	18.72	3.5
Preconditioned		
Distiller	17.30	7
Ale	16.95	14

Table II. Concentrations of higher alcohols in the fermentation distillates (µg/mL). Concentration of higher alcohols after distillation of a malt wort fermented with cells of the distilling yeast strain of *S. cerevisiae* M-type and the ale strain *S. cerevisiae* NCYC 1681 preconditioned (or not) with zinc.

	Methanol	n-Propanol	n-Butanol	2-Methyl-1-butanol	3-Methyl-1-butanol	Isobutanol	Total
Control							
Distiller	7.1	248	10.6	125.5	417	169.4	977.6
Ale	5.8	164	8.3	112.6	405	152.7	848.4
Preconditioned							
Distiller	10.1	236	11.2	126.2	439	169.0	991.5
Ale	6.2	118	4.3	121.8	465	186.6	901.9

Table III. Concentrations of secondary products in the fermentation distillates (µg/mL). Concentration of esters after distillation of a malt wort fermented with cells of the distilling yeast strain of S. cerevisiae M-type and the ale strain *S. cerevisiae* NCYC 1681 preconditioned (or not) with zinc.

	Acetaldehyde	Ethyl acetate	Acetal	Iso-amyl acetate	Furfural	Total
Control						
Distiller	723	85.1	59.7	0.6	1.5	869.9
Ale	575	48.7	52.0	0.6	1.9	678.2
Preconditioned						
Distiller	521	96.2	40.7	0	1.1	659
Ale	358	34.7	27.3	0	3.0	423

dary fermentation metabolites identified in this study, esters are the most important aroma compounds in fermented beverages, although at high concentrations they can be detrimental to the flavour and aroma. Around 100 distinct esters have so far been identified in beer[2]. Like higher alcohols, they are mainly produced during the initial stages of fermentation[10]. They are formed by the esterification of the co-enzyme acetyl co-A by alcohols, mainly ethanol, but also by some other higher alcohols. This reaction is catalysed by alcohol acetyltransferase. Levels of ethyl acetate in the un-preconditioned ale yeast strain distillates were higher (+40%) compared with Zn-preconditioned cultures. The distiller's yeast produced the highest levels of ethyl acetate regardless of zinc. No isoamyl acetate was produced in any of the Zn-preconditioned distillates. Although this compound, generated by the reaction between 3-methyl-1-butanol and acetyl co-A, is found at very low concentrations in distilled spirits, it is important in authenticity tests of Scotch whisky.

In the ale and distiller's yeast strains, the concentrations of acetal and acetaldehyde were both higher in the control distillates compared with the Zn-preconditioned distillates. Acetaldehyde is the most important aldehyde produced by fermenting yeast, as it is the penultimate intermediate in the production of ethanol. Not all acetaldehyde is converted to ethanol by the enzyme alcohol dehydrogenase, however, and some may also be excreted from the cells into the medium. This may indicate that zinc preconditioning of the yeast strains enhances the conversion of acetaldehyde to ethanol by stimulating alcohol dehydrogenase. However, the final concentrations of ethanol produced by ale and distiller's strains were consistently higher in cultures that were not zinc-preconditioned. This could be explained by zinc-stimulated decreases in acetaldehyde concentrations due to the reversion back to pyruvate and subsequent conversion into various α-keto-acids, intermediates of higher alcohols. Thus, the increase in some of the higher alcohols produced in the Zn-preconditioned distillates of the ale and distiller's yeast strains may be a result of the reversion of acetaldehyde to pyruvate and eventually to higher alcohols. It may also be possible that the α-keto-acids formed vicinal diketones (not measured in this study), which were excreted from the cell into the growth medium.

CONCLUSIONS

Zinc accumulation by a whisky distilling yeast strain of *Saccharomyces cerevisiae* and the impact of zinc preconditioning on congener formation after distillation were studied in this research. In malt wort, zinc accumulation by the distilling yeast employed was very fast. This resulted in a dramatic decrease in zinc concentration from the medium with zinc depletion observed within 30 min. Zinc accumulation by yeast in synthetic defined medium was affected by various carbohydrates. Maltose enhanced zinc accumulation at zinc concentrations above 3.2 μg/mL and fructose at zinc concentrations below 1.6 μg/mL.

As shown in this study, zinc influences yeast physiology and particularly impacts on the production of volatile flavour congeners. The latter relies on individual conditions employed by distilled beverage producers with regard to fermentation conditions, wort composition and choice of yeast strain. In industry, every effort is made to keep these parameters as consistent as possible in order to produce a more uniform product. The potential use of zinc preconditioned yeast in distillery fermentations may provide benefits for specific flavour profiles in the final distillate. In addition, zinc preconditioning may be used to augment the production of defined alcohols during fermentation by stimulating key yeast enzymes.

A panel of different malt worts and scale-up of the experiments described in this study need to be carried out to confirm the quantitative findings of this work and to better represent the conditions used in production of distilled beverages. However, this research has clearly demonstrated that zinc plays important roles in influencing distilling yeast fermentation performance and final product quality.

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