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ORIGINAL RESEARCH

Influence of heat shock-treated cells on the production of glycerol and other metabolites in alcoholic fermentation

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Abstract: The impact of heat shock on the formation of sensorily important fermentation metabolites was investigated. Initially the heat tolerance of six commercial *Saccharomyces cerevisiae* yeast strains was evaluated under various conditions of time and temperature (heat shock at 40°C, 50°C, and 60°C for a duration of 20, 40, and 60 minutes, respectively). A chemically defined grape juice medium was inoculated from the surviving colonies, and microferments were conducted. Two strains were selected for further evaluation due to their heat shock tolerance and enhanced glycerol production. The experiment was repeated in standard laboratory scale fermentations under aerobic and anaerobic conditions, and the medium was inoculated directly after the heat shock treatment and after recovery from the heat shock on yeast peptone dextrose plates. All fermentations were further analyzed for higher alcohol, organic acid, and ethyl ester content using gas chromatography mass spectrometry. Elevated glycerol production (increase of 17% under aerobic conditions and 8% under anaerobic conditions) was reported only in one strain and only after direct inoculation of the fermentation medium. With both strains, direct inoculation of the heated cells caused a 2-day delay in the commencement of the fermentation, but after recovery, the fermentation progress was increased. Volatile analysis showed that apart from changes in organic acids, all other volatile compounds analyzed exhibited an alteration mainly due to strain differences and the presence of oxygen.

Keywords: heat shock, glycerol, higher alcohols, wine, *Saccharomyces*

Introduction

Under various shock conditions yeast are known to alter the production of secondary metabolites as part of the response to such challenges. Among other compounds, glycerol has been reported to be produced in increased amounts following heat shock treatment.[1–4] In wines, glycerol has the potential to influence sensory properties; however, it is not tasted until present in excess of 5.2 g/L, and increases in viscosity are not apparent below 25 g/L.[5,6] Apart from glycerol, no other compounds of sensory importance have been reported to be produced after heat shock application.

After ethanol and carbon dioxide, glycerol is the most important by product of the alcoholic fermentation.[6–8] It is found in wines at a minimum concentration of 4.2 g/L and up to 15–20 g/L depending on the fermentation conditions.[8,9] Accumulation of glycerol and other polyols acts as a mechanism of osmotic regulation by the yeast cell when grown in a high osmolarity environment.[10] However, under nonstress conditions, the main function of glycerol is to act as a route for the regeneration of NAD^+ from NADH, and thereby maintains intracellular redox balance.[3,11,12]

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Alcohols with more than two carbon atoms are known as higher alcohols. They are produced during all alcoholic fermentations and are quantitatively the largest group of aroma compounds in alcoholic beverages.[13] Their concentration in wines varies from slightly less than 100 mg/L to higher than 500 mg/L.[14–16] They are formed by yeast either aerobically from sugars or from the catabolism of grape amino acids via the Ehrlich reaction.[16–20] Through these reactions, the amino acids are catabolized for their nitrogen component, and the carbon skeletons have alternative fates. The resultant α-keto acids may be excreted directly or decarboxylated to the corresponding aldehyde and then reduced to the alcohol.[18] Research conducted reported that yeasts form higher alcohols because at the final reduction step NADH is reoxidized to $NAD,^{+}$ and thus, helps to maintain the redox balance of the cell.[21–23] Alternatively, in other research, it was stated that the exact function of higher alcohol formation is unknown, and the reaction is a minor route for NADH reoxidation.[19] Instead, it is suggested that higher alcohol formation may simply serve to detoxify any aldehydes produced during amino acid catabolism or may be involved in the regulation of amino acid anabolism.

The enhanced production of glycerol under heat shock conditions is already reported by many authors.[1–4] The purpose of this research is to investigate the possibility of applying prefermentation heat shock not only to achieve increased glycerol production but also to prevent the loss of cell viability, so as not to cause problems in fermentation kinetics and the commencement/completion of the alcoholic fermentation. Furthermore, this research aimed to evaluate the impact of the heat shock on the production of secondary metabolites. This was the first research investigating the impact of heat shock on the production of ethyl esters, higher alcohols, acetates, and acids. The heat treatments applied are near lethal for yeast, unlike with previous research conducted by other authors, where the heat treatment was moderate.[1–4] Regardless of the physiological basis, the production of higher alcohols, ethyl esters, and glycerol could have important implications for beverage fermentations and could be a tool for winemakers and brewers in achieving improved quality.

Materials and methods

Yeast strains and preliminary evaluation of heat response

Six commercial strains of active dried wine yeast *Saccharomyces cerevisiae* were selected due to their ability to produce high levels of glycerol according to claims made by the manufacturers: QA23, L2056, M69 (Lallemand, South Australia, Australia) and AWRI 796, AWRI R2, and PDM (Maurivin, Toowoomba, Australia). Isolates of each were obtained from commercially available preparations and liquid cultures in yeast peptone dextrose (YPD; yeast extract, 10 g/L; peptone, 20 g/L; and dextrose, 20 g/L) stored at −80°C following the addition of glycerol to 15% (w/v). Fresh YPD cultures (0.6 mL in a polymerase chain reaction tube) were subject to various heat treatments in a thermocycler (20, 40, and 60 minutes at 40°C, 50°C, and 60°C, respectively), and the cells streaked onto solid YPD (20 g/L agar) and incubated at 30°C for 3 days before inspection. Colonies recovered from these plates were then evaluated in microscale fermentations. Thus, selected colonies were grown overnight at 30°C in 1 mL of YPD in wells of a 48-well plate, before a 48-pin replicating tool was used to inoculate triplicate experimental cultures containing 1 mL of chemically defined grape juice medium (CDGJM).[24] Daily the fermentation rate was monitored using a hand-held refractometer (°Brix; Reichert, Depew, NY). Although convenient, this approach did not allow the end of the fermentation to be defined exactly since the volume of the fermentations did not offer sufficient sample for the more accurate determination of residual sugars eg, by enzymatic assay. Therefore, the end of the fermentations was designated the point 2 days after a refractometer reading of 6°Brix was obtained. Previous experience showed that the end of the ferment coincides with that point.

At the conclusion of fermentation, cultures were centrifuged (3,500 rpm, 10 minutes; Eppendorf, Hamburg, Germany), and the clarified samples were stored at −20°C. The glycerol content of the fermented medium was determined enzymatically (Boehringer Mannheim/R-Biopharm, Darmstadt, Germany).

Experimental cultures

Two strains of *S. cerevisiae* (QA23 and L2056) were selected for further study since they exhibited the greatest difference in glycerol production in the preliminary evaluation. Subsequent work was limited to these strains and the extreme heat treatment at which surviving cells were still recovered (60°C for 20 minutes). To apply the heat treatment, the yeast strains were grown overnight in YPD broth, adjusted to 1×10^{8}/mL, heated, and the viable cell number determined by methylene blue staining.[25] Triplicate experimental cultures were conducted in 100 mL of CDGJM inoculated with 5×10^{6} viable cells/mL taken either directly from the heat treatment or following overnight growth in YPD. Fermentations were grown in both aerobic and anaerobic conditions with agitation

(160 rpm) at a standard temperature of 30°C. Anaerobic conditions were maintained by using flasks fitted with a silicone bung coupled with air trap.

Analytical methods

Fermentation rate was monitored by refractive index (°Brix) and completion was confirmed using Clinitest® tablets (Bayer, Leverkusen, Germany) following the manufacturer's instructions. After the end of the fermentation, yeast dry cell weight was determined, and culture supernatants were stored at −20°C prior to glycerol determination (Boehringer Mannheim/R-Biopharm) and quantitation of relevant metabolites. Ethyl ester, acetate, higher alcohol, and organic acid concentration were determined as described previously.[26] Briefly, a Hewlett Packard 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA) was equipped with a Gerstel MPS2 multipurpose sampler and coupled to a HP 5973N mass selective detector. The instrument was controlled, and the data were analyzed with HP G1701CA ChemStation software. The gas chromatograph was fitted with a 60 m × 0.25 mm J&W fused silica capillary column (DB-Wax, 0.25 µm film thickness). The carrier gas was ultra-high purity helium at a linear velocity of 36 cm/s and flow rate of 2.0 mL/min in constant flow mode. The oven was started at 40°C, held for 4 minutes, then increased to 220°C at 5°C/min and held for 20 minutes. The inlet was fitted with a borosilicate glass PME inlet liner (0.75 mm I.D; Supelco, Supelco Park, PA) and was held at 200°C. The solid-phase microextraction (SPME) fiber was desorbed in the pulsed splitless mode, and the splitter, at 25:1, was opened after 30 seconds. The fiber was allowed to bake in the inlet for 10 minutes. The mass spectrometer quadrupole temperature was set at 106°C, the source set at 230°C, and the transfer line held at 250°C. Positive-ion electron impact spectra at 70 eV were recorded in selective ion monitoring.

The conditions for headspace SPME sampling were as follows: a 10 mL aliquot of diluted sample (1 in 10 dilution in Milli Q water; Millipore, Billerica, MA) was added to a 20 mL vial containing 2 g of sodium chloride, and the vial immediately crimp-capped. Subsequently, 100 µL of combined internal standard solution was injected through the septum, and the vial was shaken well. The vial and its contents were heated to 35°C. The Supelco Carbowax/divinylbenzene 65 µm fiber was exposed to the headspace for 10 minutes. To accommodate volatiles that might be found in higher concentrations, the wine sample was diluted 1 in 100 rather than 1 in 10 as described above.

Results

Initial evaluation of heat shock treatment on wine yeast

Following exposure to a range of temperatures for various durations (Heat shock at 40°C, 50°C, and 60°C for duration of 20, 40, and 60 minutes, respectively), the heat-shocked cells were streaked onto triplicate YPD plates and incubated at 30°C for 3 days. Plates were then examined for the degree of colony formation, with differences being evident between strains. A subset only of these findings is reported in Table 1. Apart from there being differences between strains, the general and expected trends were described; ie, that at higher temperatures and longer exposure times, a decreasing proportion of the yeast cultures were able to develop into colonies on YPD plates. No colonies developed from populations treated at 60°C for 60 minutes. Strain L2056 exhibited the greatest tolerance of the treatments and was the only strain to yield a colony from a culture sample held for 40 minutes at 60°C (Table 1). By comparison, QA23 appeared to only tolerate this temperature for 20 minutes.

For all six strains, a colony which had developed on the YPD plates after each heat treatment was used to establish a culture (1 mL) in YPD broth in a 48-well plate (160 rpm, 30°C; overnight). These were then inoculated in triplicate into 1 mL of CDGJM using a 48-pin replicating tool and incubated as above. Supernatants were assayed for glycerol content (Figure 1). The control cultures (no heat shock) showed glycerol yields ranging from 100 mg/L to approximately 5 g/L. Although some treatments resulted in lower glycerol production, many of the heat shock treatments produced an increase in glycerol yield. In most cases, such increases were modest; however, for L2056, glycerol yields increased by around 10-fold to nearly 6 g/L for the 60°C treatment. Based on these results, further work was performed with strains L2056, a high producer and highly responsive strain,

Table 1 Preliminary evaluation of sensitivity of commercial wine yeasts L2056 and QA23 to a range of heat shock treatments as judged from relative growth of heat-shocked cultures on yeast peptone dextrose plates after incubation at 30°C for 3 days

Exposure (min)	L2056			QA23		
	40°C	50°C	60°C	40°C	50°C	60°C
20	+++	++	±	++++	+++	+
40	++	++	±	+++	++	–
60	+++	+	–	+++	++	–

Note: Growth is defined as maximum (+++), moderate (++), low (+), trace (±), or absent (–).

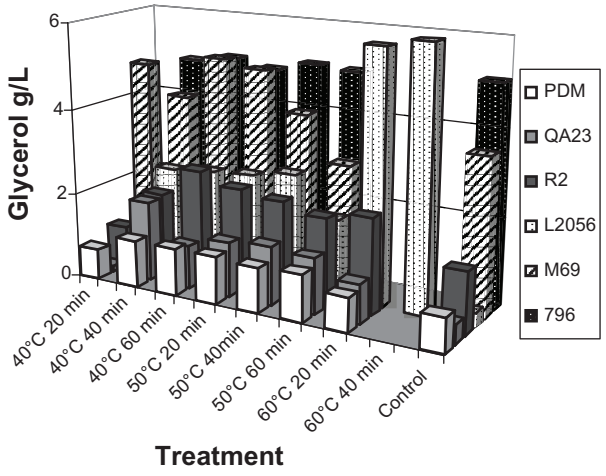

Figure 1 Glycerol content of microfermentations by six commercial wine yeast strains after heat shock treatment at various times and temperatures.
Notes: Only L2056 was able to survive treatment at 60°C for 40 minutes. Two histograms for the 40°C, 20 minute treatment are obscured: strain L2056 (0.06 g/L) and strain 796 (0.89 g/L). Values are the mean of triplicate fermentations. Standard deviations of the means were <10%.

and QA23, which produced more modest yields of glycerol (Figure 1).

Laboratory scale fermentations of heat shock-treated cells

Following the initial evaluation of heat treatment, a more comprehensive evaluation was undertaken. In this case, fermentations were conducted at the laboratory scale in volumes of 100 mL, and fermentation progress was monitored so as to provide a more comprehensive view of the response to the heat shock. For this purpose, triplicate fermentations were inoculated to 5×10^6 cells/mL from the same plates that were used for inoculating the microfermentations, and incubation was conducted under both aerobic and anaerobic conditions. Although there were minor differences in fermentation duration (data not shown), yields of glycerol by L2056 and QA23 were not different between the controls and the treated cells (data not shown). For this reason and as an attempt to reflect a more likely scenario for application of a heat-shock in the winery, treated cultures were evaluated immediately after heat treatment. Thus, overnight YPD cultures of strains L2056 and QA23 were exposed to 60°C for 20 minutes before determination of viability (methylene blue) and inoculation of triplicate aliquots of 100 ml of CDGJM to 5×10^6 viable cells/mL. To allow comparison with the aerobic conditions used in the microfermentations, both aerobic and anaerobic conditions were used.

Fermentations by heat-shocked L2056 exhibited an initial lag of about 2 days and a total fermentation time of 6 days (Figure 2). By comparison, the control cultures commenced fermentation with no apparent delay but slowed such that they completed in the same time (6 days) as the treated cells under aerobic conditions or 1 day earlier (5 days) under anaerobic conditions. Similar delays in commencement of fermentation were seen for QA23 in both aerobic and anaerobic conditions; however, the control culture completed fermentation in each case 1 day earlier than the treated cultures.

Although the results from the microfermentations exhibited glycerol yields ranging from 0.8 to 5.8 g/L for the 60°C/20 min treatment across all 6 strains, the impact of heat treatment was not as obvious in the larger scale fermentations (Table 2). Even so, the greater tendency of L2056 to produce glycerol was reaffirmed with yields consistently above 5 g/L compared with values between 3.4 and 4.9 g/L for QA23. These data also indicate that L2056 was able to increase glycerol production by between 9% and 20% following heat treatment, whereas QA23 was not. The impact of heat treatment on the generation of other metabolites, particularly volatiles of potential sensory significance, was subsequently examined.

Gas chromatography mass spectrometry analysis of fermentation volatiles

Quantification of glycerol after heat treatment was intended as a possible indicator of altered metabolite yields has been shown by brewing researchers.[2,27] Gas chromatography mass spectrometry analysis was required to more completely evaluate chemical differences among strains and heat treatments. The chemical compounds selected for detection were chosen according to their sensory importance and available methods for quantitation. Thus, included groups of compounds were the ethyl esters, acetates, alcohols, and acids, with these being determined in the same samples used to generate the glycerol data above. Differences were evident between the aerobic and the anaerobic with 2- and 3-methyl butanoic acid, decanoic acid, ethyl hexanoate, hexanoic acid, 2-methyl propanoic acid, butanoic acid, and octanoic acid concentrations being markedly lower in the anaerobic cultures for both strains (Table 3). Beyond these, selected compounds differed in their content between the control and heat-shocked cells depending on strain. For L2056, heat shock reduced phenlyethyl acetate content under both oxygen availabilities, whereas 2-methyl propan-1-ol was

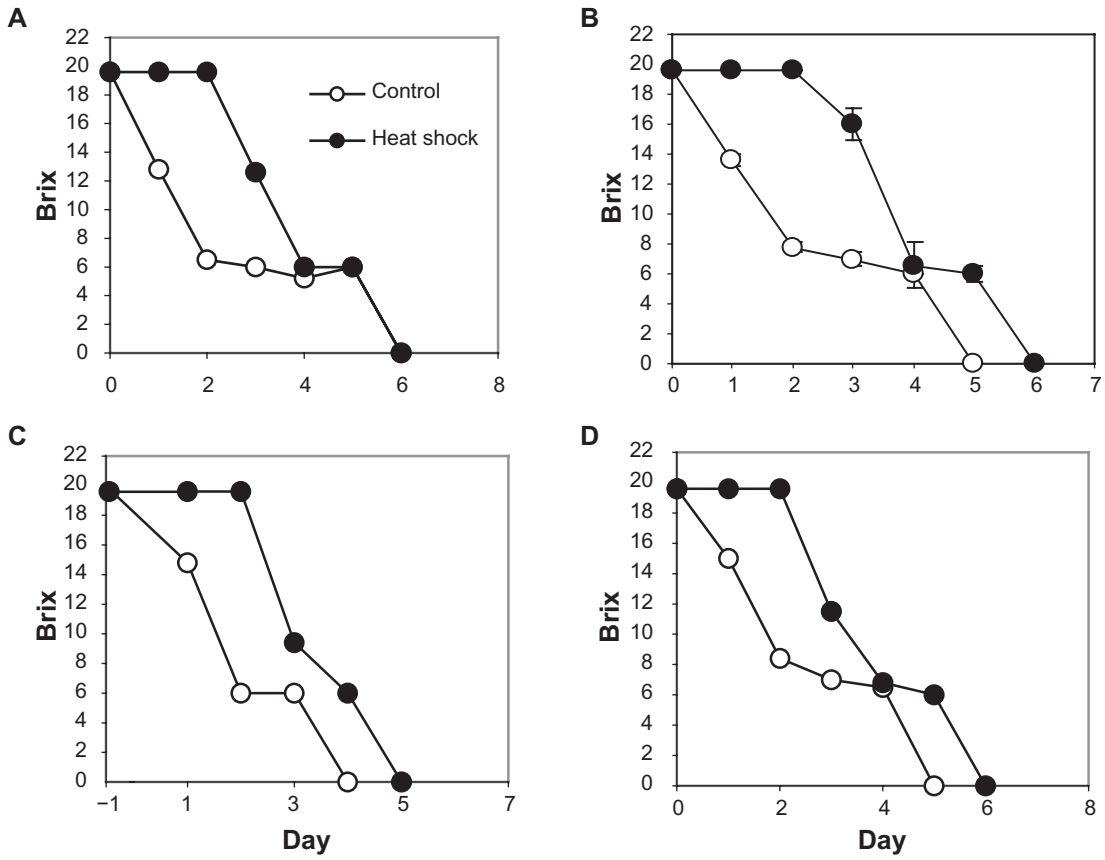

Figure 2 Fermentation progress (Brix vs time) of lab scale (100 ml) fermentations inoculated directly after heat shock treatment (60°C/20 min) of yeast strain L2056 (top) and QA23 (bottom) and incubated under aerobic (**A** and **C**) or anaerobic conditions (**B** and **D**).
Notes: Control was not heat shocked. Values are the mean of triplicate fermentations. Error bars indicate standard deviation and in most cases are too small to be seen behind the symbols (see B for visible error bars).

increased in the presence of air. Also, for L2056, hexanoic and butanoic acids were reduced in aerobic conditions upon heat shock, and 2-methyl propan-l-ol, 2-methyl butanoic acid, 2-methyl propanoic acid, octanoic acid, ethyl butanoate, phenyl ethylacetate, and propanoic acid were decreased in anaerobic conditions.

In the case of QA23, 2-methyl propan-1-ol, propanoic acid, and ethyl lactate were reduced upon heat shock under aerobic conditions, whereas the same occurred anaerobically for 3-methyl butyl acetate, decanoic acid, ethyl acetate, hexanoic acid, octanoic acid, propanoic acid, ethyl butanoate, ethyl dodecanoate, ethyl 2-methyl propanoate, and ethyl propanoate. The concentration of a number of compounds increased in aerobic culture following heat shock, including for example, 2-methylbutyl acetate, 2-methyl butanoic acid, 3-methyl butanoic acid and ethyl acetate.

Table 2 Glycerol content (g/L) of lab scale fermentations after heat shock treatment (60°C/20 min) of yeast strains L2056 and QA23 and incubation under aerobic and anaerobic conditions. The control had no treatment

Strain	Incubation conditions	Control	Heat shock
L2056	Aerobic	5.4 ± 0.1	6.5 ± 0.6
	Anaerobic	6.7 ± 0.3	7.3 ± 0.3
QA23	Aerobic	3.5 ± 0.2	3.4 ± 0.1
	Anaerobic	4.9 ± 0.4	4.9 ± 0.6

Note: Values are the mean of triplicate fermentations ± standard deviation.

Discussion

The data reported here show broad divergence in terms of the sensitivity and response of yeast strain to heat shock. In both strains selected for closer investigation (QA23 and L2056), exposure of the yeast cells to extreme temperatures caused an expected gradual decrease in the population. However, the surviving cells exhibited a delay in the commencement of the fermentation also reported by other researchers.[27–29] Such delays might be attributable to cell death or reduced fermentative activity that took some time to manifest itself, or alterations in metabolism due to heat shock delay, culture development, and fermentation.[28] Further, the methylene blue viability determination may have been an underestimate of

Table 3 Metabolite yields by control and heat-shocked (60°C/20 min) cultures of L2056 (standard font) and QA23 (italics) grown in 100 mL fermentations of chemically defined grape juice medium under either aerobic or anaerobic conditions

Analyte	Aerobic conditions		Anaerobic conditions	
	Control	Heat shock	Control	Heat shock
2-Methylbutyl acetate (μg/L)	ND	ND	46.70 ± 0.5	52.60 ± 37.2
	37.90	*ND*	*32.70 ± 2.0*	*38.90*
Phenylethyl acetate (μg/L)	137 ± 1.6	46.20 ± 7.3	180 ± 1.9	139 ± 0.8
	52.50 ± 2.3	*52.90 ± 0.1*	*51.50 ± 2.2*	*51.60 ± 0.5*
3-Methylbutyl acetate (μg/L)	111 ± 2.5	122 ± 15	131 ± 1.5	153 ± 3.1
	70.10 ± 7.0	*92.10*	*106.6 ± 1.8*	*51.60*
2-Phenylethan-1-ol (mg/L)	22.92 ± 0.25	22.92 ± 0.46	27.76 ± 0.20	30.41 ± 0.77
	19.53 ± 0.27	*19.17 ± 0.29*	*23.67 ± 0.98*	*26.24 ± 0.11*
3-Methylbutan-1-ol (mg/L)	85.23 ± 4.95	79.84 ± 2.86	75.14 ± 4.48	70.49 ± 5.40
	105.76 ± 8.04	*92.695 ± 1.60*	*85.25 ± 7.31*	*105.07 ± 11.67*
2-Methyl butanol (mg/L)	41.85 ± 1.48	41.14 ± 0.59	43.04 ± 0.26	42.97 ± 0.25
	50.02 ± 0.89	*50.44 ± 0.95*	*52.03 ± 0.78*	*54.88 ± 1.86*
2-Methyl propan-1-ol (mg/L)	45.45 ± 0.81	49.81 ± 0.75	40.98 ± 1.56	34.06 ± 0.55
	48.29 ± 1.18	*38.97 ± 5.31*	*47.12 ± 2.31*	*51.04 ± 6.93*
Propanoic acid (μg/L)	6,256 ± 440	5,930 ± 352	2,501 ± 67.7	ND
	3,986 ± 1,147	*2,530 ± 86*	*3,202 ± 84*	*2,471 ± 1,557*
2-Methyl butanoic acid (μg/L)	1,952 ± 57	1,750 ± 21	1,034 ± 4.3	932 ± 37
	1,996 ± 150	*2,119 ± 36*	*1,376 ± 41*	*1,597 ± 27*
Hexanoic acid (μg/L)	391 ± 49	270 ± 13	10.5 ± 8.5	5.6
	1,204 ± 19	*1,207 ± 5.0*	*445 ± 7.4*	*386 ± 5.2*
2-Methyl propanoic acid (μg/L)	5,028 ± 168	5,004 ± 68	2,120 ± 120	1,672 ± 166
	7,572 ± 283	*7,471 ± 33*	*4,932 ± 42*	*5,202 ± 116*
3-Methyl butanoic acid (μg/L)	926 ± 26	826 ± 53	419 ± 39	424 ± 67
	869 ± 72	*924 ± 4*	*639 ± 20*	*755*
Butanoic acid (μg/L)	1,724 ± 94	1,417 ± 8	802 ± 17	950 ± 54
	2,062 ± 175	*1,929 ± 16*	*1,060 ± 128*	*917 ± 42*
Decanoic acid (μg/L)	470 ± 34	414 ± 11	247.7 ± 22.75	220 ± 2
	517 ± 15	*376 ± 20*	*333 ± 17*	*150 ± 11*
Octanoic acid (μg/L)	1,291 ± 57	1,260 ± 4	636.1 ± 0.4	556 ± 30
	1,202 ± 71	*1,203 ± 12*	*546 ± 36*	*438 ± 29*
Ethyl acetate (mg/L)	30.58 ± 0.06	32.82 ± 0.14	28.92 ± 0.11	29.38 ± 0.23
	37.36 ± 0.07	*45.69 ± 0.11*	*38.67 ± 0.40*	*24.09 ± 0.22*
Ethyl butanoate (μg/L)	23.40 ± 1.0	32.90 ± 1.2	57.30 ± 2.9	52.10 ± 0.1
	30.80 ± 0.6	*57.60*	*57.30 ± 1.3*	*40.50 ± 1.8*
Ethyl hexanoate (μg/L)	62.40	83.10 ± 12	26.50 ± 4.3	59.50 ± 4.0
	54.80 ± 13.3	*73.50*	*23.70 ± 3.2*	*20.80*
Ethyl dodecanoate (μg/L)	16.30 ± 0.5	ND	16.70 ± 0.8	23.80 ± 2.0
	15.60 ± 2.4	*23.40 ± 0.5*	*43.60 ± 3.0*	*31.30 ± 0.8*
Ethyl propanoate (μg/L)	75.10 ± 0.4	73.90 ± 4.8	62.80 ± 2.2	58.10 ± 2.8
	96.80 ± 4.5	*127*	*78.20 ± 3.4*	*56.10 ± 0.7*
Ethyl 2-methyl butanoate (μg/L)	3.10 ± 0.1	3.70 ± 0.2	2.90 ± 0.2	2.10 ± 0.1
	2.70 ± 0.4	*3.40*	*2.20 ± 0.2*	*1.70 ± 0.1*
Ethyl lactate (μg/L)	873 ± 6	925 ± 58	768 ± 2	867 ± 57
	864 ± 36	*741 ± 4*	*751 ± 35*	*776 ± 43*
Ethyl 2-methyl propanoate (μg/L)	19.40 ± 0.3	25.80 ± 1.4	16.70 ± 0.2	14.70 ± 1.6
	21.70 ± 1.5	*32*	*15.70 ± 0.6*	*10.50 ± 0.1*
Ethyl octanoate (μg/L)	8.50 ± 0.3	8.90 ± 1.9	4.80 ± 0.1	5.60 ± 0.7
	17.50 ± 6.3	*9.80 ± 3.9*	*15.80 ± 5.9*	*8.50 ± 6.1*

Notes: Values are the mean of triplicate fermentations ± standard deviation. In some cases poor peak resolution resulted in only two replicates (and therefore, no SD) being available.

Abbreviations: ND, not detected; SD, standard deviation.

the proportion of viable cells, therefore, resulting in a lower inoculation rate. However, the fact that heat-shocked cultures typically recovered and attained a faster fermentation rate than the controls, implies an enduring consequence of heat treatment, as has been shown previously.[1,2]

In our experiment, increases in the glycerol production after the heat shock were reported only when using yeast strain L2056 in the case of the direct inoculation (Table 2), whereas in the case of intermediate growth on plates, production remained the same as the unheated control (data not shown). Experiments conducted with yeast strain QA23 did not present the same behavior, and net difference in the glycerol yield was independent of the inoculation of the medium when conducted directly after the heat shock (Table 2) or after growth on plates (data not shown). An explanation for there being no net change in glycerol concentration after the growth of the heat shocked cells on plates could be the gradual loss of the heat shock response and any associated production of protectant compounds such as glycerol.[29] These findings, therefore, suggest that for at least one strain, heat shock prior to inoculation may increase glycerol yield and, apparently, robustness of the inoculum, thereby permitting a higher fermentation rate. Trials on a larger scale to determine efficacy of this treatment under commercial conditions are therefore warranted.

Higher alcohol composition in wines can be affected by the yeast strain and the must.[30,31] The results from the fermentation trials reported here indicate that the heat treatment also altered the yield of several volatile compounds as shown for brewing strains.[2] However, these changes in volatile compound concentration, which were observed among strains and heat shock conditions, were nonuniform. Apart from changes in organic acids, all other volatile compounds analyzed exhibited an alteration mainly due to strain differences and due less to the influence of the heat shock. The production of higher alcohols was below the threshold levels reported in wine and beer, eg, the aroma threshold of 3-methylbutane-1-ol in wine is reported at 300 mg/L.[20] However, only 70–106 mg/L was detected in the heat shock experiments described above. Nevertheless, changes in overall concentration of fermentation products related to heat shock were observed, and further research to clarify this impact is warranted.

Anaerobic growth caused elevated production of glycerol independent of the strain or the timing of inoculation following the heat shock. These findings are in agreement with other researchers who also reported enhanced glycerol production under anaerobic conditions.[32] Production of higher alcohols is reported to be enhanced under aerobic conditions.[33,34] However, this was not confirmed under the experimental conditions described above.

Differences in the glycerol content were observed between microferments and the laboratory scale fermentations. There is a potential that due to the small volume of the fermentation medium, microscale ferments could exhibit evaporation, altering the concentration of the chemical compounds. Therefore, although this technique allows for rapid screening of a large number of samples, further clarification of the results obtained is required, as highlighted by the subsequent fermentation studies we performed.

Glycerol, esters, and higher alcohols are key determinants of the sensory properties of wines. Their presence in high quantities is usually indicative of quality. However, most of the factors that enhance the formation of one compound act negatively on the formation of another compound (ie, must clarification positively affects the formation of esters but has the opposite function on the production of higher alcohols). Our findings showed that heat treatment resulted in an increase on the glycerol content of the wine; however, the response of heat-shocked yeast to the formation of other sensory important chemical compounds was variable and influenced by the strain investigated.

Conclusion

Heat shock treatment is a simple and easy method that could enhance the formation of sensory important metabolites such as glycerol, and therefore, might be used to alter the sensory properties of wine. The temperature and the duration of the heat shock that would be used commercially needs to be better defined and tailored for individual yeast strains, and should ideally not result in a decrease of the yeast population.

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Disclosure

The authors report no conflicts of interest in this work.

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