

# Evaluation of the acetaldehyde production and degradation potential of 26 enological *Saccharomyces* and non-*Saccharomyces* yeast strains in a resting cell model system

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**Abstract** Acetaldehyde is relevant for wine aroma, wine color, and microbiological stability. Yeast are known to play a crucial role in production and utilization of acetaldehyde during fermentations but comparative quantitative data are scarce. This research evaluated the acetaldehyde metabolism of 26 yeast strains, including commercial *Saccharomyces* and non-*Saccharomyces*, in a reproducible resting cell model system. Acetaldehyde kinetics and peak values were highly genus, species, and strain dependent. Peak acetaldehyde values varied from 2.2 to 189.4 mg l<sup>-1</sup> and correlated well ( $r^2 = 0.92$ ) with the acetaldehyde production yield coefficients that ranged from 0.4 to 42 mg acetaldehyde per g of glucose in absence of SO<sub>2</sub>. *S. pombe* showed the highest acetaldehyde production yield coefficients and peak values. All other non-*Saccharomyces* species produced significantly less acetaldehyde than the *S. cerevisiae* strains and were less affected by SO<sub>2</sub> additions. All yeast strains could degrade acetaldehyde as sole substrate, but the acetaldehyde degradation rates did not correlate with acetaldehyde peak values or acetaldehyde production yield coefficients in incubations with glucose as sole substrate.

**Keywords** Acetaldehyde · *Saccharomyces cerevisiae* · Non-*Saccharomyces* · Resting cells · Yield coefficient

## Introduction

Acetaldehyde (ethanal) is a potent volatile flavor compound found in many beverages and foods [20]. In wines, it contributes to red wine color [32, 33, 37] and is responsible for the special flavor of certain wines, such as sherry and port [8, 12]. However, in most finished wines, acetaldehyde is undesired because of its unpleasant, grassy, or oxidized aroma [7, 23] and its capacity to strongly bind with SO<sub>2</sub> [35], which has antimicrobial and antioxidant roles. More recently, acetaldehyde has been suggested to be a direct cause of alcoholic beverage-derived carcinogenicity, too [17, 18].

Biological acetaldehyde formation mainly results from the activity of yeast during early phases of the alcoholic fermentation where acetaldehyde serves as terminal electron acceptor [24, 34]. Acetaldehyde may be reutilized during later fermentation stages by yeast. It is known that the ability to produce acetaldehyde is a yeast strain-dependent trait [5, 13, 21, 30, 38], but the physiological differences among commercial *Saccharomyces cerevisiae* starters and the increasingly popular non-*Saccharomyces cerevisiae* strains with regards to acetaldehyde have not been systematically evaluated. Liu and Pilone [20] reviewed the literature with regards to the range of acetaldehyde levels produced by 10 different yeast species including non-*Saccharomyces*, but the values were derived from experiments with differing fermentation conditions. Romano [31] investigated the acetaldehyde production ability of several *S. cerevisiae* strains in synthetic medium and in grape must, but the study only measured final acetaldehyde concentrations, which highly depend on the fermentation conditions and the possible formation of acetaldehyde from chemical oxidation of ethanol [39].

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The aim of this study was to compare the acetaldehyde production and degradation potential of 26 yeast strains including currently available commercial *S. cerevisiae* and non-*Saccharomyces* strains. The evaluation was carried out with time course studies applying a highly reproducible model system using a synthetic buffer and resting yeast cells.

## Materials and methods

### Microorganisms

*Metschnikowia pulcherrima* C6, *Zygosaccharomyces bailii* C23, *Candida stellata* C35, *Hansenula anomala* C4, *Candida vini* C2, *Hanseniaspora uvarum* C1, and *Schizosaccharomyces pombe* C7 were taken from the culture collection of the Laboratory for Wine Microbiology at the Department of Food Science and Technology of Cornell University (Geneva NY, USA). Isolates of the commercial starters of *Saccharomyces cerevisiae* CY3079, DV10 and EC1118 were obtained from Lallemand Inc. (Montreal, Quebec, Canada). Sixteen further *S. cerevisiae* strains were isolated from spontaneous fermentations of wines in the Xinjiang wine region of China by the College of Enology of the Northwest A&F University (Yangling, Shaanxi, China) and identified to species level using biochemical profiling (API 20C AUX kit, bioMérieux, Marcy l'Etoile, France).

### Culture conditions and cell harvest

Yeasts were grown aerobically in YPD broth (Fisher, Hanover Park, IL, USA) in 250-ml shake cultures at 28°C (Innova 5000 orbital shaker, New Brunswick, NJ, USA) to early stationary phase as measured by dry weight [19]. After cell harvest by centrifugation at 5,000g for 10 min at 15°C, the cell sediment was washed twice with buffer (7.5 g tartaric acid per liter deionized water, adjusted to pH 4.0 with NaOH). Cell sediments were then resuspended into the same buffer and their dry weight adjusted by appropriate dilution followed by reassessment of the dry weight. Finally, 20-ml aliquots of yeast cell suspensions were added to 22-ml glass flat-bottomed vials sealed with a rubber stopper for subsequent resting cell experiments.

### Resting cell experiments

Resting cell experiments were performed according to Osborne et al. [28] with modifications. The glass vials containing yeast suspensions were placed in a water bath and stirred with Teflon-coated stir bars (4 × 1 mm) at 800 rpm using a submersible magnetic stirrer (2mag-USA,

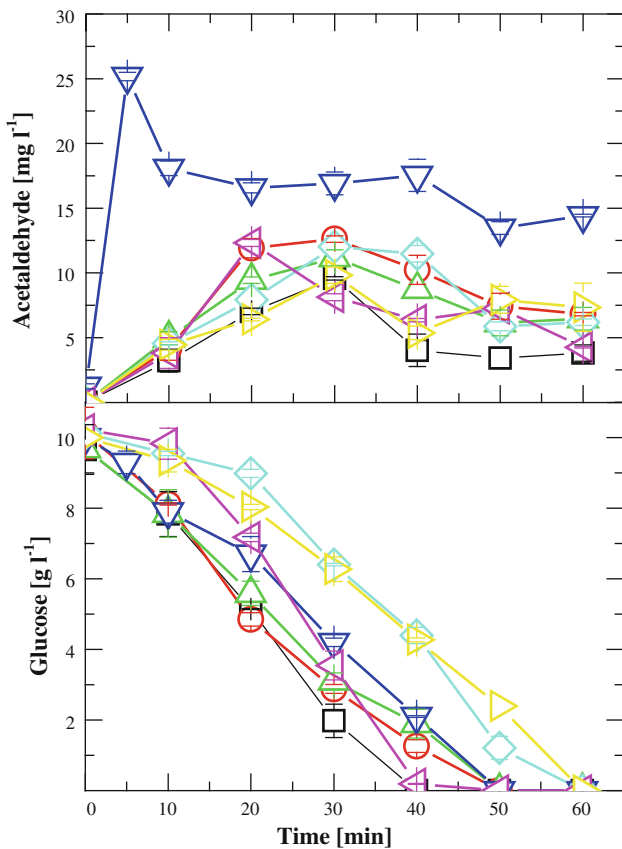
Daytona Beach, FL, USA). For acetaldehyde production experiments, appropriate volumes of a highly concentrated glucose stock solution (500 g l<sup>-1</sup>) were added to cell suspensions adjusted to 15 g dry weight l<sup>-1</sup>. Incubations with SO<sub>2</sub> were realized by adding appropriate volumes of a 50 g l<sup>-1</sup> SO<sub>2</sub> stock solution prepared by dissolving 8.675 g of potassium metabisulfite in water and adjusting the volume to 100 ml. For acetaldehyde degradation experiments, acetaldehyde was adjusted to 80 mg l<sup>-1</sup> and yeast biomass was adjusted to 7.5 g dry weight l<sup>-1</sup>. During incubations, samples were taken periodically with syringes through the rubber seal of closed vials, cooled in an ice salt bath (−18 to −20°C), and centrifuged at 10,000g for 5 min. Finally, the supernatant was recovered and stored at −20°C for subsequent analysis.

### Analytical methods and statistical analysis

Acetaldehyde was measured enzymatically with a commercial test kit (Megazyme, UK). Glucose was measured by HPLC using a Shimadzu Prominence system (Columbia, MD, USA). Following injection of 3 µl previously filtered samples (0.22 µm, nylon membrane, Whatman, NJ, USA) and separation on an ion exchange column at 85°C (RHM Monosaccharide H + 8%, 300 × 7.8 mm, Phenomenex, Torrance, CA, USA) glucose was quantified by refractive index (RID-10A, Shimadzu Tokyo, Japan). The mobile phase was 100% water and the flow rate was 0.5 ml min<sup>-1</sup>. Data representation and rate fittings were carried out using Origin v7.0 (OriginLab, Northampton, MA, USA) and statistical analysis was carried out using SPSS v.16 (Chicago, IL, USA).

## Results

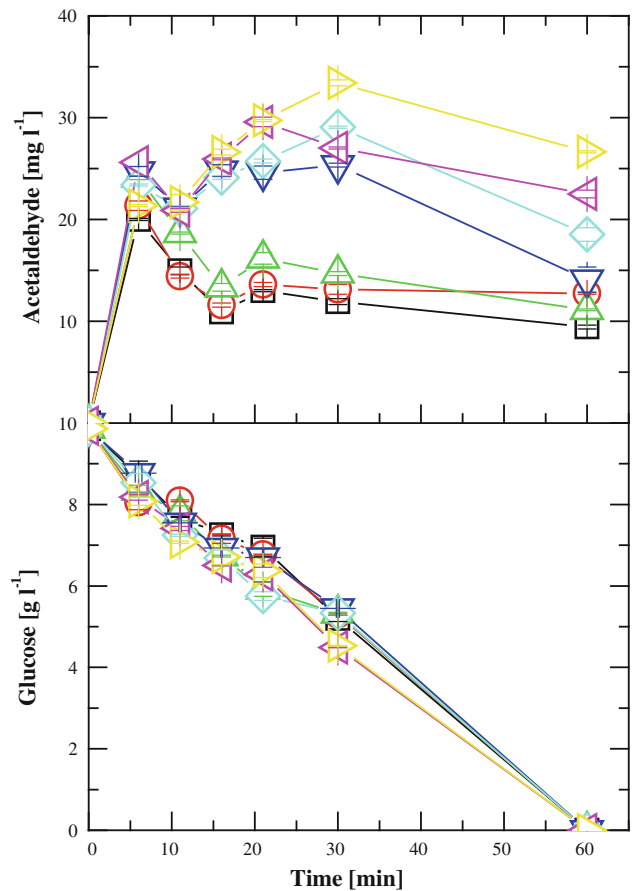
Acetaldehyde kinetics of *Saccharomyces* and non-*Saccharomyces* strains were first investigated in resting cell suspensions at pH 3.6 and with 10 g l<sup>-1</sup> initial glucose. Figure 1 shows results from this experiment. For all strains, acetaldehyde concentrations increased at the beginning of incubations until reaching a peak, after which concentrations decreased again. The rates of increase and decrease as well as the time point where the peak occurred and its concentration were strain dependent (Fig. 1). *S. pombe* displayed the highest acetaldehyde formation rate and peak value and was used to further optimize the method with regards to medium pH, temperature, and initial glucose concentration in order to select incubation conditions that would allow one to effectively discriminate between strains while simulating must conditions. Figure 2 illustrates the effect of buffer pH on acetaldehyde kinetics. While not influencing the glucose degradation rate significantly,



**Fig. 1** Course of glucose and acetaldehyde concentrations during yeast resting cell experiments with  $10 \text{ g l}^{-1}$  initial glucose. The buffer pH was 3.6 and yeast biomass was adjusted to  $10 \text{ g l}^{-1}$  of dry weight. Yeast species:  $\square$ , CY3079;  $\circ$ , DV10;  $\triangle$ , EC1118;  $\nabla$ , *S. pombe*;  $\diamond$ , *H. uvarum*;  $\blacktriangleleft$ , *H. anomala*;  $\blacktriangleright$ , *C. vini*. Data shows the average of duplicate incubations  $\pm$ SE (standard error)

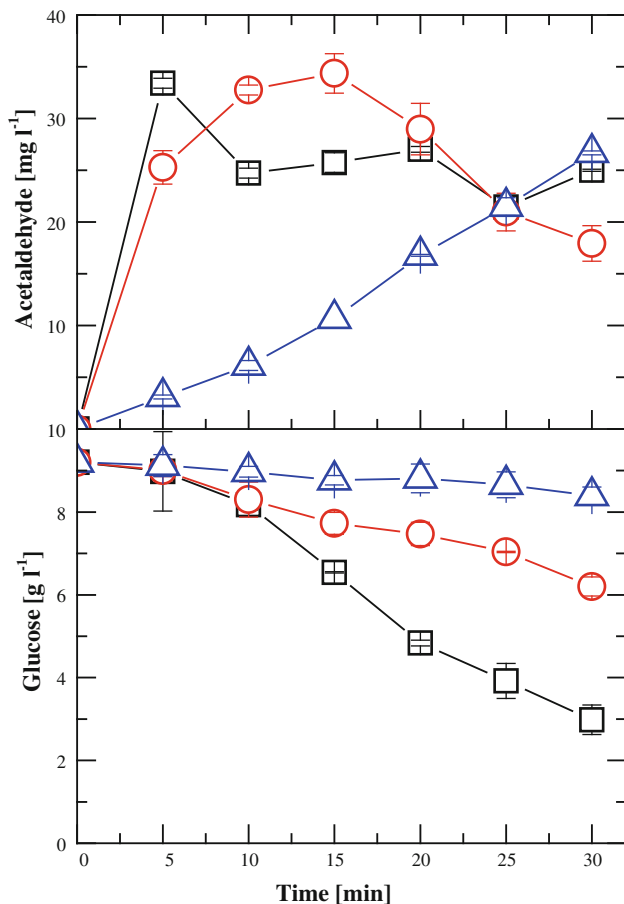
higher pH values led to increased acetaldehyde peak and final concentrations. Figure 3 shows the effect of the fermentation temperature. Higher temperatures accelerated the glucose degradation rates, which were associated with increased acetaldehyde accumulation rates. However, no statistically significant difference was obtained among the acetaldehyde production yield coefficients (average amounts of acetaldehyde produced per glucose degraded) from incubations at three different temperatures ( $n = 2$ ,  $P = 0.05$ ). Figure 4 demonstrates the influence of the initial glucose concentration on acetaldehyde kinetics during resting cell experiments with *S. pombe*. The total amount of glucose degraded and the initial acetaldehyde production rate were similar in all treatments. However, the total amount of acetaldehyde produced and the peak values depended highly on the initial sugar concentration.

On the basis of these results, all further incubations were conducted at pH 4.0, a temperature of  $30^\circ\text{C}$ , and  $200 \text{ g l}^{-1}$  of initial glucose. The yeast biomass



**Fig. 2** Effect of buffer pH on glucose and acetaldehyde concentrations during resting cell experiments with *S. pombe*. pH values:  $\square$ , 3.0;  $\circ$ , 3.2;  $\triangle$ , 3.4;  $\nabla$ , 3.6;  $\diamond$ , 3.8;  $\blacktriangleleft$ , 4.0;  $\blacktriangleright$ , 4.2. Yeast biomass was adjusted to  $7.5 \text{ g l}^{-1}$  of dry weight. Initial glucose concentration was  $10 \text{ g l}^{-1}$ . Data display average of duplicate incubations  $\pm$ SE

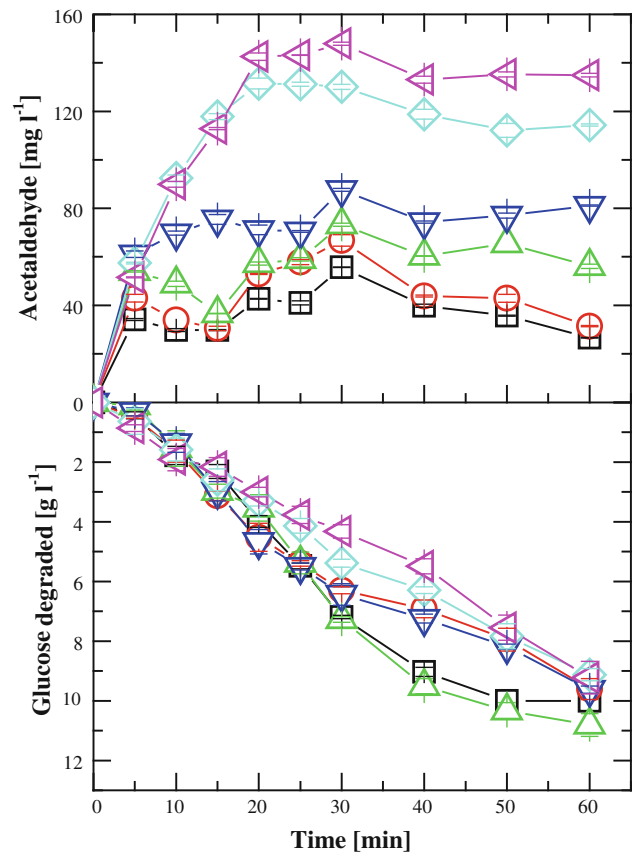
concentrations were adjusted to  $15 \text{ g l}^{-1}$  dry weight. The method developed was then applied to the study of acetaldehyde formation and degradation kinetics. Results obtained by using the optimized method were highly reproducible leading to average coefficients of variation of 8.38% and 1.36% across all tested strains for the acetaldehyde production yield coefficient and the peak values, respectively. The acetaldehyde production yield coefficients and peak values were extracted from the experimental results and were found to be highly genus, species, and strain dependent (Table 1). The acetaldehyde production yield coefficients ranged from 0.4 to  $42 \text{ mg acetaldehyde per g of glucose}$ , and peak acetaldehyde values ranged from  $2.2$  to  $189.4 \text{ mg l}^{-1}$  in resting cell experiments without  $\text{SO}_2$  addition. A good linear relationship was found between the acetaldehyde production yield coefficient and peak values in experiments without and with addition of  $50 \text{ mg l}^{-1}$  of  $\text{SO}_2$  ( $r^2$  values obtained were 0.92 and 0.91, respectively). The highest peak values were obtained in experiments with *S. pombe* ( $189 \text{ mg l}^{-1}$ ).



**Fig. 3** Effect of fermentation temperature on glucose and acetaldehyde concentrations during resting cell experiments with *S. pombe*. Temperature:  $\square$ , 30°C;  $\circ$ , 20°C;  $\triangle$ , 10°C. Buffer pH was 4.0 and yeast biomass was adjusted to 7.5 g l<sup>-1</sup> of dry weight. Initial glucose concentration was 10 g l<sup>-1</sup>. Data display average of duplicate incubations  $\pm$ SE

*S. cerevisiae* strains led to intermediate peak values ranging between 23 and 100 mg l<sup>-1</sup> with an average of 67 mg l<sup>-1</sup>. Except for *S. pombe*, the non-*Saccharomyces cerevisiae* yeast strains produced the lowest acetaldehyde peak ranging from 2 to 40 mg l<sup>-1</sup> with an average of 20 mg l<sup>-1</sup> (Table 1).

Initial addition of SO<sub>2</sub> led to higher peak acetaldehyde levels in all strains and the differences were of statistical significance for all but two of the strains. There was also a trend for higher acetaldehyde yield coefficients in the presence of SO<sub>2</sub> in all but two cases. The extent of peak acetaldehyde increases from SO<sub>2</sub> addition was highly strain dependent and ranged from 31 to 587  $\mu$ g acetaldehyde per mg of SO<sub>2</sub> added with an average increase of 278  $\mu$ g acetaldehyde per mg SO<sub>2</sub> (Table 1). The highest overall increase was evident in *S. cerevisiae* strains, which displayed average increases of 328  $\mu$ g acetaldehyde per mg of SO<sub>2</sub> with minima and maxima of 31 and 587, respectively. An intermediate increase was found for *S. pombe*



**Fig. 4** Effect of initial glucose concentration on acetaldehyde and glucose kinetics during resting cell experiments with *S. pombe*. Glucose levels:  $\square$ , 10;  $\circ$ , 20;  $\triangle$ , 50;  $\nabla$ , 100;  $\diamond$ , 200;  $\hexagon$ , 240 g l<sup>-1</sup>. Buffer pH was 4.0 and yeast biomass was adjusted to 7.5 g l<sup>-1</sup> of dry weight. Data display average of duplicate incubations  $\pm$ SE

(337  $\mu$ g acetaldehyde mg of SO<sub>2</sub>) and the lowest overall increase could be observed for other non-*Saccharomyces cerevisiae* strains (58–155  $\mu$ g acetaldehyde per mg of SO<sub>2</sub> with an average of 112, Table 1).

The degradation of externally supplied acetaldehyde in the absence of sugar was also investigated in separate incubations. The values were obtained from the initial acetaldehyde degradation rate and normalized for biomass. Figure 5 shows an example of the acetaldehyde degradation curves obtained for three yeast strains. The initial acetaldehyde degradation rates of 26 strains calculated from such graphs are shown in Table 1. Resting cells of all 26 yeast strains were able to degrade the externally added free acetaldehyde in the absence of sugar in a strain-dependent manner. *S. pombe* showed the lowest acetaldehyde degradation rate (0.04 mg g<sup>-1</sup> min<sup>-1</sup>). All other non-*Saccharomyces* yeast strains displayed degradation rates of 0.24–0.50 mg g<sup>-1</sup> min<sup>-1</sup>, whereas *Saccharomyces* strains displayed the highest values with 0.49–0.77 mg g<sup>-1</sup> min<sup>-1</sup>. No significant relationship was

**Table 1** Acetaldehyde yield coefficients and peak values as well as acetaldehyde degradation rates (separate experiments) of resting yeast cells

| Yeast strains         | Yield coefficient (mg g <sup>-1</sup> glucose) |                                       | Peak value (mg l <sup>-1</sup> ) |                                       | SO <sub>2</sub> effect on acetaldehyde peak (µg mg <sup>-1</sup> SO <sub>2</sub> ) | Degradation rate (mg g <sup>-1</sup> min <sup>-1</sup> ) |
|-----------------------|--|---------------------------------------|----------------------------------|---------------------------------------|--|--|
|                       | No SO <sub>2</sub>                             | 50 mg l <sup>-1</sup> SO <sub>2</sub> | No SO <sub>2</sub>               | 50 mg l <sup>-1</sup> SO <sub>2</sub> |  |  |
| <i>M. pulcherrima</i> | 0.38 ± 0.18 <sup>l</sup>                       | 0.52 ± 0.16 <sup>k</sup>              | 2.19 ± 0.03 <sup>q</sup>         | 5.11 ± 0.18 <sup>ts</sup>             | 58.3 ± 2.4 <sup>lm</sup>   | 0.46 ± 0.01 <sup>hi</sup>                                |
| <i>C. stellata</i>    | 1.09 ± 0.23 <sup>l</sup>                       | 1.13 ± 0.18 <sup>jk</sup>             | 21.73 ± 0.47 <sup>n</sup>        | 26.47 ± 1.59 <sup>q</sup>             | 94.8 ± 50.6 <sup>kl</sup>  | 0.38 ± 0.01 <sup>j</sup>                                 |
| <i>H. anomala</i>     | 1.06 ± 0.08 <sup>l</sup>                       | 1.18 ± 0.04 <sup>jk</sup>             | 15.52 ± 0.27 <sup>o</sup>        | 20.86 ± 0.67 <sup>r</sup>             | 106.8 ± 2.8 <sup>ikl</sup>   | 0.50 ± 0.01 <sup>h</sup>                                 |
| <i>Z. bailii</i>      | 1.04 ± 0.21 <sup>l</sup>                       | 1.09 ± 0.19 <sup>jk</sup>             | 31.85 ± 0.37 <sup>m</sup>        | 38.65 ± 0.42 <sup>p</sup>             | 135.9 ± 6.7 <sup>ijk</sup>   | 0.24 ± 0.01 <sup>k</sup>                                 |
| <i>C. vini</i>        | 1.50 ± 0.35 <sup>l</sup>                       | 1.78 ± 0.04 <sup>jk</sup>             | 8.09 ± 0.42 <sup>p</sup>         | 15.86 ± 0.66 <sup>s</sup>             | 155.4 ± 3.7 <sup>ij</sup>  | 0.25 ± 0.01 <sup>k</sup>                                 |
| <i>H. uvarum</i>      | 3.62 ± 0.08 <sup>k</sup>                       | 6.76 ± 0.18 <sup>*</sup>              | 39.79 ± 0.31 <sup>k</sup>        | 45.78 ± 0.79 <sup>o</sup>             | 119.8 ± 3.4 <sup>ijk</sup>   | 0.42 ± 0.02 <sup>ij</sup>                                |
| <i>S. pombe</i>       | 41.98 ± 1.44 <sup>a</sup>                      | 47.44 ± 0.29 <sup>*</sup>             | 189.39 ± 0.66 <sup>a</sup>       | 206.23 ± 0.96 <sup>a</sup>            | 336.8 ± 7.4 <sup>de</sup>  | 0.04 ± 0.00 <sup>1</sup>                                 |
| EC1118                | 1.81 ± 0.16 <sup>l</sup>                       | 3.17 ± 0.40 <sup>ij*</sup>            | 23.40 ± 0.31 <sup>n</sup>        | 38.27 ± 1.58 <sup>p</sup>             | 297.4 ± 44.1 <sup>ef</sup>   | 0.50 ± 0.05 <sup>h</sup>                                 |
| DV10                  | 4.49 ± 0.45 <sup>jk</sup>                      | 7.42 ± 0.12 <sup>gh*</sup>            | 35.69 ± 0.39 <sup>l</sup>        | 50.78 ± 1.58 <sup>n</sup>             | 301.8 ± 47.2 <sup>ef</sup>   | 0.62 ± 0.01 <sup>efg</sup>                               |
| CY3079                | 5.98 ± 0.44 <sup>hij</sup>                     | 6.50 ± 0.31 <sup>h</sup>              | 57.33 ± 0.66 <sup>hj</sup>       | 66.07 ± 1.31 <sup>k</sup>             | 174.7 ± 0.4 <sup>hi</sup>  | 0.68 ± 0.02 <sup>bcdef</sup>                             |
| E1809                 | 4.42 ± 0.51 <sup>jk</sup>                      | 5.22 ± 0.85 <sup>hi</sup>             | 38.26 ± 0.20 <sup>k</sup>        | 55.94 ± 0.42 <sup>m</sup>             | 353.5 ± 16.3 <sup>d</sup>  | 0.74 ± 0.01 <sup>ab</sup>                                |
| A709                  | 5.00 ± 0.14 <sup>ijk</sup>                     | 5.82 ± 0.47 <sup>h</sup>              | 49.45 ± 0.54 <sup>j</sup>        | 62.42 ± 0.60 <sup>l</sup>             | 259.3 ± 33.5 <sup>fg</sup>   | 0.77 ± 0.02 <sup>a</sup>                                 |
| E1217                 | 5.85 ± 0.18 <sup>hij</sup>                     | 7.05 ± 0.15 <sup>h*</sup>             | 67.28 ± 0.60 <sup>g</sup>        | 85.33 ± 0.93 <sup>s</sup>             | 361 ± 5.4 <sup>d</sup>   | 0.70 ± 0.02 <sup>bcd</sup>                               |
| E1215                 | 5.89 ± 0.21 <sup>hij</sup>                     | 5.58 ± 0.02 <sup>h</sup>              | 66.31 ± 0.63 <sup>g</sup>        | 77.06 ± 0.89 <sup>i</sup>             | 215.1 ± 7.2 <sup>gh</sup>  | 0.70 ± 0.03 <sup>abc</sup>                               |
| A711                  | 6.55 ± 0.25 <sup>hi</sup>                      | 6.45 ± 0.16 <sup>h</sup>              | 77.53 ± 0.29 <sup>f</sup>        | 79.10 ± 0.40 <sup>i</sup>             | 31.4 ± 3.4 <sup>m</sup>  | 0.69 ± 0.04 <sup>bcde</sup>                              |
| G511                  | 7.12 ± 0.02 <sup>h</sup>                       | 10.23 ± 0.38 <sup>fe</sup>            | 55.33 ± 0.24 <sup>i</sup>        | 62.99 ± 0.78 <sup>l</sup>             | 153.2 ± 5.9 <sup>ij</sup>  | 0.65 ± 0.03 <sup>cdefg</sup>                             |
| E1504                 | 9.06 ± 0.24 <sup>g</sup>                       | 9.38 ± 1.29 <sup>fg</sup>             | 75.58 ± 0.25 <sup>f</sup>        | 81.92 ± 0.75 <sup>h</sup>             | 126.8 ± 25.2 <sup>ijk</sup>  | 0.61 ± 0.04 <sup>fg</sup>                                |
| P509                  | 9.17 ± 0.47 <sup>g</sup>                       | 11.25 ± 0.89 <sup>ef</sup>            | 80.81 ± 0.47 <sup>e</sup>        | 110.15 ± 0.95 <sup>ef</sup>           | 586.8 ± 0.3 <sup>a</sup>   | 0.63 ± 0.06 <sup>cdefg</sup>                             |
| E1219                 | 9.74 ± 0.16 <sup>g</sup>                       | 10.01 ± 0.23 <sup>f</sup>             | 58.91 ± 0.16 <sup>h</sup>        | 71.09 ± 0.77 <sup>j</sup>             | 243.5 ± 21.9 <sup>g</sup>  | 0.67 ± 0.03 <sup>bcdef</sup>                             |
| N524                  | 10.07 ± 1.19 <sup>g</sup>                      | 10.61 ± 0.45 <sup>f</sup>             | 81.80 ± 1.21 <sup>e</sup>        | 108.70 ± 1.63 <sup>fs</sup>           | 537.9 ± 15.7 <sup>b</sup>  | 0.66 ± 0.05 <sup>cdef</sup>                              |
| P525                  | 12.30 ± 0.35 <sup>f</sup>                      | 16.36 ± 0.11 <sup>d*</sup>            | 99.86 ± 0.48 <sup>b</sup>        | 126.20 ± 1.11 <sup>b</sup>            | 526.8 ± 2.8 <sup>b</sup>   | 0.58 ± 0.01 <sup>g</sup>                                 |
| N518                  | 12.67 ± 0.39 <sup>ef</sup>                     | 12.97 ± 0.84 <sup>e</sup>             | 50.56 ± 0.90 <sup>j</sup>        | 57.37 ± 0.67 <sup>m</sup>             | 136.2 ± 49.5 <sup>ijk</sup>  | 0.70 ± 0.06 <sup>abc</sup>                               |
| R312                  | 14.01 ± 1.00 <sup>e</sup>                      | 18.57 ± 3.63 <sup>c</sup>             | 90.17 ± 0.51 <sup>c</sup>        | 113.54 ± 1.48 <sup>cd</sup>           | 467.4 ± 9.3 <sup>c</sup>   | 0.63 ± 0.05 <sup>defg</sup>                              |
| P529                  | 16.05 ± 0.64 <sup>d</sup>                      | 18.22 ± 1.02 <sup>cd</sup>            | 92.20 ± 0.47 <sup>c</sup>        | 114.42 ± 1.10 <sup>c</sup>            | 444.3 ± 40.6 <sup>c</sup>  | 0.50 ± 0.01 <sup>h</sup>                                 |
| P504                  | 17.76 ± 2.20 <sup>c</sup>                      | 20.16 ± 0.78 <sup>c</sup>             | 82.23 ± 0.54 <sup>e</sup>        | 108.26 ± 1.55 <sup>fs</sup>           | 520.6 ± 9.3 <sup>b</sup>   | 0.63 ± 0.03 <sup>cdefg</sup>                             |
| P522                  | 20.02 ± 1.63 <sup>b</sup>                      | 24.19 ± 2.68 <sup>b</sup>             | 87.28 ± 0.80 <sup>d</sup>        | 111.99 ± 0.50 <sup>des</sup>          | 494.1 ± 21.9 <sup>bc</sup>   | 0.49 ± 0.04 <sup>h</sup>                                 |

Yield coefficients were calculated as average amounts of acetaldehyde produced per glucose degraded during the initial acetaldehyde production phase. Data display average of duplicate incubations ±SE

Superscript letters display statistically significant differences of average values obtained for yeast strains in a column (*P* < 0.05)

\* Displays statistically significant differences of average yield coefficient values obtained for one yeast between the treatment with or without SO<sub>2</sub> (*P* < 0.05)

§ Displays statistically significant differences of average peak acetaldehyde values obtained for one yeast between the treatment with or without SO<sub>2</sub> (*P* < 0.05)

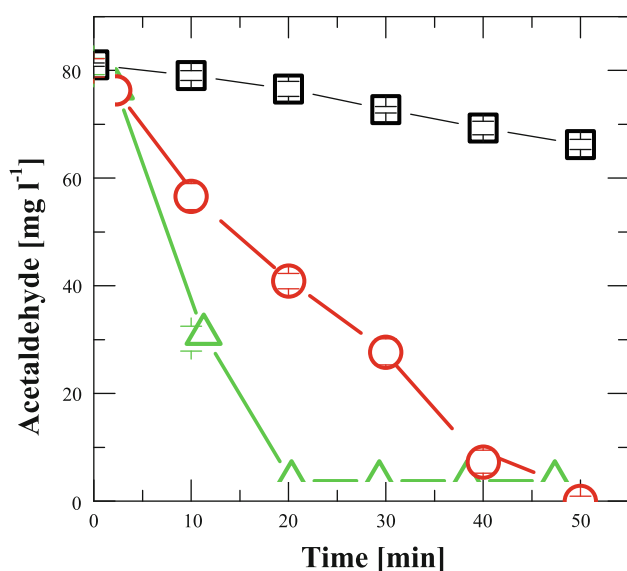
found between acetaldehyde production yield coefficients or peak values and the degradation rates on the basis of the results of this study.

**Discussion**

Acetaldehyde is a by-product and terminal electron acceptor during alcoholic fermentations (AF) by yeast. SO<sub>2</sub>, the major wine preservative, strongly binds to acetaldehyde essentially removing it as electron acceptor from AF, and leading to increased acetaldehyde production by yeast as a result [25, 26]. It is known that acetaldehyde

production among wine yeast is strain specific [20, 36] and several studies have suggested to survey yeast acetaldehyde production and to include this trait among yeast strain selection parameters [6, 31]. However, no data quantifying acetaldehyde production among *Saccharomyces* and non-*Saccharomyces* wine yeast under comparable and easily reproducible conditions are available. Resting cell suspensions in a winemaking buffer system have been used in the past to study the metabolism of wine yeast and lactic acid bacteria providing results similar to fermentations in must, but in a fraction of the time required [22, 28]. After standardized pre-growth of the yeast and adjustment of the biomass using a rapid method [19], resting cell incubations





**Fig. 5** Courses of acetaldehyde degradation during yeast resting cell experiments.  $\square$ , *S. pombe* C7;  $\circ$ , *Z. bailii* C23;  $\triangle$ , *S. cerevisiae* CY3079. Yeast biomass was adjusted to  $7.5 \text{ g l}^{-1}$  of dry weights. Buffer pH was 4.0. Data display average of duplicate incubations  $\pm$ SE

only lasted between 0.5 and 2 h in this study, and the data obtained were highly reproducible. Both the kinetics of acetaldehyde production and degradation, as well as peak and final values were within the range of values reported from fermentations in musts and wines [15, 16, 20, 27]. In this study, final acetaldehyde values were not considered for the assessment of the yeast acetaldehyde production potential because they highly depended on yeast viability and vitality during the latter phases of incubations. These effects may also account for the wide range of values reported in the literature for final acetaldehyde levels, in addition to potential formation by chemical oxidation of ethanol [39]. Instead, acetaldehyde yield coefficients (acetaldehyde produced per glucose degraded) as well as peak values were quantified.

The enological parameters pH, temperature,  $\text{SO}_2$  addition, and initial glucose concentration were initially varied in order to both reflect the wine environment and to maximize the ability of the method to effectively discriminate among yeast strains with regards to their phenotype. Increased buffer pH values increased acetaldehyde peak and final concentrations by resting cells of *S. pombe* in accordance with results obtained by Delfini and Formica [9] in a synthetic medium and hence a value of 4.0 was chosen. Reports on the influence of the fermentation temperature on the acetaldehyde formation have been controversial. Romano [31] reported that final acetaldehyde concentration in wines at the point of sugar depletion was not affected by temperatures of 12, 18, and  $24^\circ\text{C}$ , whereas it increased considerably at  $30^\circ\text{C}$ . By contrast, Cabranes

[4] found a higher final acetaldehyde concentration at  $12^\circ\text{C}$  than at  $18^\circ\text{C}$  in a cider fermentation. In our work, increasing fermentation temperature from 10 to  $30^\circ\text{C}$  accelerated the glucose degradation rate and promoted the acetaldehyde production rate, but there was no significant influence on the acetaldehyde yield coefficient, i.e., the formation of acetaldehyde per glucose degraded. Because of this, the higher temperature was chosen to reduce the incubation time. In this study, increased initial sugar concentrations did not change sugar degradation rates, but significantly higher acetaldehyde peak values were obtained at the highest levels tested, which were also similar to must conditions, and hence a sugar concentration of  $200 \text{ g l}^{-1}$  was selected for the survey. After this optimization, the method was applied to the study of all yeast strains and provided good reproducibility.

A recent study analyzing eight *S. cerevisiae* strains did not find a correlation between early acetaldehyde production rates and acetaldehyde peak values [5]. Growing yeast were used in a chemically defined medium but the active dry yeast formulations, whose exact composition is not known, may have introduced additional nutrients. Also,  $\text{CO}_2$  release as assessed by weight loss was considered for the calculation of acetaldehyde production rates by Cheraiti et al. [5], but the actual quantity of the substrate glucose degraded was used to calculate acetaldehyde production yield coefficients in the current study. These differences may account for the discrepancy in the results obtained. In this study, the excellent correlation between acetaldehyde yield coefficients and the acetaldehyde peak values suggest one may consider either value for the assessment of the microbial acetaldehyde production potential. However, accurately assessing peak values requires high sampling frequencies. Hence, calculating yield coefficients using data from the initial degradation of glucose and production of acetaldehyde may prove advantageous.

This study also investigated whether strong acetaldehyde production was correlated with weak uptake of acetaldehyde as single substrate in separate experiments. A good correlation would have allowed one to utilize a simpler experimental setup to evaluate the acetaldehyde production potential. In this study, *S. pombe*, which showed the highest acetaldehyde peaks and yield coefficients, displayed the lowest ability to degrade acetaldehyde as sole substrate. However, there was no clear relationship between degradation of acetaldehyde as a sole substrate and the acetaldehyde peaks and yield coefficients obtained in incubations with glucose for all other yeast. Consequently, results obtained here do not support utilization of acetaldehyde degradation data to evaluate the acetaldehyde production potential of enological yeast.

All yeasts reacted to the addition of SO<sub>2</sub> by increasing acetaldehyde production. Although this was anticipated [25, 26], the 19-fold difference encountered between the increase of acetaldehyde peak values in the yeast least affected by the SO<sub>2</sub> addition and the most susceptible yeast was surprising. Considering these differences, the SO<sub>2</sub> susceptibility of the acetaldehyde production potential of commercial starters may serve as a strain selection argument, especially where high SO<sub>2</sub> musts are to be inoculated.

There is an increasing interest in the industrial application of non-*Saccharomyces* yeasts. Fleet [14] reviewed the enological relevance of non-*Saccharomyces* yeasts and mixed and pure lyophilized strains are now commercially available. Bely et al. [2] and Diaz-Montano et al. [11] found non-*S. cerevisiae* strains including *Torulaspota delbrueckii*, *Kloeckera africana*, and *Kloeckera apiculata* to produce lower acetaldehyde levels in grape must and agave fermentations, respectively. In the present study, strains of *M. pulcherrima*, *Z. bailii*, *C. stellata*, *H. anomala*, and *C. vini* had lower acetaldehyde yield coefficients and peak values compared with *S. cerevisiae* strains, whose acetaldehyde production capacities were relatively homogenous. In addition, the acetaldehyde production by non-*Saccharomyces* yeast was also less susceptible to SO<sub>2</sub> additions. *S. pombe* has been utilized in must fermentation to decrease malic acid concentrations, but higher final wine acetaldehyde levels went along with its cultivation [10]. In this study, too, *S. pombe* led to the highest acetaldehyde concentrations resulting in peak values of over 200 mg l<sup>-1</sup> in the presence of 50 mg l<sup>-1</sup> of SO<sub>2</sub>.

Numerous *S. cerevisiae* and few non-*Saccharomyces cerevisiae* strains are commercially available to induce AF, having been selected on the basis of various wine quality and process criteria [3, 29]. The yeast acetaldehyde production and degradation potential is not generally used as a selection criterion, yet. However, these properties can have important implications for winemaking. Low acetaldehyde producing strains may allow manufacturers to better satisfy consumer demands for lower SO<sub>2</sub> concentrations and legal limits in some legislation, such as in the EU [1]. Strains with a strong acetaldehyde degradation potential may be used within a biological fining approach after AF to reduce final bound-SO<sub>2</sub> concentrations in high acetaldehyde containing wines. Those with high acetaldehyde production potential may be conducive to color stabilization [33] in the vinification of weakly colored red varieties without the need for oxygenation.

Although formation and degradation kinetics as well as peak acetaldehyde levels are in agreement with values obtained in musts and wines [15, 16, 20, 27, 31], further work will benefit from the validation of the method presented here in model and natural grape musts.

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