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Novel Method To Reduce Fishy Aftertaste in Wine and Seafood Pairing Using Alcohol-Treated Yeast Cells

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Supporting Information

ABSTRACT: "Fishy aftertaste" is sometimes perceived in wine consumed with seafood. Iron in wine has been reported to be a key compound that produces fishy aftertaste. However, cost-effective methods to remove iron from wine have not been developed. Here, we describe a cost-effective and safe iron adsorbent consisting of alcohol-treated yeast (ATY) cells based on the observation that nonviable cells adsorbed iron after completion of fermentation. Treatment of cells with more than 40% (v/v) ethanol killed them without compromising their ability to adsorb iron. Drying the ATY cells did not reduce iron adsorption. Use of ATY cells together with phytic acid had a synergistic effect on iron removal. We term this means of removing iron the "ATY−PA" method. Sensory analysis indicated that fishy aftertaste in wine-seafood pairings was not perceived if the wine had been pretreated with both ATY cells and phytic acid.

KEYWORDS: *Fishy aftertaste, iron, wine, grape juice, alcohol-treated, yeast, phytic acid, seafood*

INTRODUCTION

Matching of wine and food plays an important role in the enjoyment of meals[1] and is a topic of great interest among wine-drinking consumers.[2,3] However, an incompatible combination has been recognized between a reduction in sweetness and umami in beverages and enhancement of sourness, bitterness, and astringency.[4] For example, pairing red wine with seafood is not generally recommended, because of the perception of unpleasant flavors, e.g., ferrous taste,[5] fishy and metallic odors, and bitterness as a result of tannins in wine.[6] However, because white wines, which usually have little tannins, can also clash with fish,[7] this recommendation has been based on anecdotal or empirical evidence. A recent study has shown that iron, at concentrations commonly found in wine, is a key factor in the formation of the aforementioned unpleasant fishy aftertaste in wine−seafood pairings with both red and white wines.[8]

According to a 2009 report of the United Nations Food and Agriculture Organization (FAO), apparent world per capita fish (seafood) consumption has been increasing steadily, from an average of 11.5 kg in the 1970s to 12.5 kg in the 1980s to 14.4 kg in the 1990s and reaching 16.4 kg in 2005. The most dramatic increase has occurred in east Asia (mainly in China). In Japan, per capita fish (seafood) consumption per year was approximately 4 times greater than the world average in 2005.[9] Because a typical Asian meal consists of a bowl of rice with a variety of side dishes, it is not practical to imagine matching a single wine with an Asian meal.[10] A preferable solution would be a selection of versatile wines that paired well with a wide range of flavors.[10]

The average concentration of iron in wine worldwide has been reported to range from 2.8 to 16 mg/L[11] and to be about 23 mg/L in grape juice.[12] Iron in wine and juice is derived largely from soil, dust, and processing equipment.[13] In general, reducing iron concentrations to less than 4 mg/L in wine minimizes the risk of Fe-associated cloudiness, oxidation, discoloration, metallic taste, and metal toxicity.[14,15] Excessive iron concentrations can be reduced by use of specific fining agents or cooling to induce precipitation. However, the very low levels of iron in wine that cause fishy aftertaste cannot be removed by conventional methods, including ion exchange,[16,17] chemical precipitation or adsorption using activated carbon, or chelating resin,[18,19] without affecting other compounds. This is because most wines contain many divalent cations (e.g., calcium, magnesium, and zinc) that will compete for removal by ion exchange and chelating resins, making the process costly. While chemical precipitation is a relatively inexpensive alternative, it is restricted by safety considerations. For example, ferrocyanide is probably the most efficient method to remove iron, but its use poses toxic waste disposal problems.[20,21] Phytic acid, which has been used most frequently as a metal adsorbent in food processing,[22,23] has also been used to remove iron from wine and grape juice generally as calcium phytate. The International Organization of Vine and Wine (OIV) recommends use of calcium phytate as an iron-removal agent,[24] while Trela reported that a molar ratio of 1:1 phytic acid/iron and 5:1 calcium/phytic acid reduced iron concentrations by >90%.[21] However, as Trela also reported, phytic acid was not effective in all wines and juices and did not reduce iron levels sufficiently (to less than a few milligrams per liter) to prevent fishy aftertaste, possibly because of calcium levels already present in wine or the extent of aeration.[21]

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Because of these problems, a satisfactory method for removing iron from wine to reduce fishy aftertaste in wine−seafood pairings has not yet been achieved. Here, we report that nonviable yeast cells harvested late in fermentation were able to adsorb iron from red and white wines. On the basis of this observation, we describe a practical method for removing iron from wine using yeast as an adsorbent.

MATERIALS AND METHODS

Yeast Strains and Fermentation. The yeast strains used in this study are listed in Table 1. Yeast cells were grown in YPD (1% yeast extract, 2% peptone, and 2% glucose), YPD10 (1% yeast extract, 2% peptone, and 10% glucose), or grape juice for making white wine in our laboratory. Generic white grape juice concentrate from Argentina (68 ± 2 °Brix) was dissolved and diluted to 20 °Brix with distilled deionized ultrapure water and supplemented with 0.1% diammonium phosphate as a nitrogen source. The iron concentration of the diluted juice was measured and adjusted to 4−7 mg/L using ferrous sulfate heptahydrate.

Table 1. List of Strains Used in This Study

strain	species	source
SYT001	*Saccharomyces cerevisiae*	laboratory strain[25]
OC-2	*Saccharomyces cerevisiae*	Brewing Society of Japan
DV10	*Saccharomyces cerevisiae*	Lalvin
Dia yeast	*Saccharomyces cerevisiae*	Product of Kirin Kyowa Foods Co., Ltd.
IFO1127	*Saccharomyces bayanus*	Institute for Fermentation, Osaka, Japan
IFO1167	*Saccharomyces pastorianus*	Institute for Fermentation, Osaka, Japan

The wine yeast OC-2 was precultured statically at 25 °C for 3 days in 30 mL of YPD10. Cells were harvested and diluted to an optical density (OD_{600}) of 1.0 in 30 mL of 20 °Brix grape juice, as described above. The initial iron concentration in the grape juice was 4.38 mg/L. Optical density was measured spectrophotometrically (GeneQuant 1300, GE Healthcare) using a correction factor of 0.18. Fermentation was performed statically at 20 °C for approximately 30 days in a 50 mL test tube in the presence of oxygen. Samples were withdrawn during fermentation in triplicate to assess sugar and yeast concentrations and yeast viability. The sugar content was measured using a density meter (DMA4500, Anton Parr).

Wines. Wines (wine D) used for sensory analysis were made from diluted grape juice described above. Active dry yeast DV10 was rehydrated according to the protocol of the manufacturer and added to 10 L of grape juice. Fermentation was performed statically at 20 °C for approximately 7 days in a 15 L jar fermentor. After completion of the fermentation, the wine was centrifuged and clarified using 50 mg/L chitosan and 300 mg/L bentonite as fining agents. Other white wines (wines A and B) and red wine (wine C) were obtained from the domestic market in Japan. Profiles of the coded samples are shown in Table 2. Titratable acidity was analyzed as tartaric acid. Sulfur dioxide and the pH of wines were analyzed by the Ripper determination method and the standard pH procedure, respectively.

Table 2. Profiles of Wine Samples

sample	alcohol (%, v/v)	titratable acidity (g/L as tararic acid)	pH	free SO_2 (mg/L)	total SO_2 (mg/L)
wine A	13.3	6.8	3.2	35	90
wine B	11.5	4.5	3.1	44	148
wine C	11.6	4.7	3.0	38	150
wine D	11.3	4.8	3.0	39	157

Measurement of Cell Viability. Yeast cell viability was measured by the oxonol [bis-(1,3-dibutylbarbituric acid) trimethine oxonol, DiBAC4(3)] staining method (Invitrogen), followed by flow cytometric analysis, as reported using a FACSort cytometer (Becton-Dickinson).[26] The threshold was set on forward scatter (FSC) at 253 V. FSC and side scatter (SSC) detector voltages were E-1 and 273 V, respectively. Fluorescence detectors were adjusted to 551 V.

Yeast slurry samples (10 μL) were diluted into 1 mL of 10 ng/mL oxonol solutions. The solutions were vortex-mixed (5 s), incubated (10 min), and further vortex-mixed (10 s) prior to flow cytometric analysis. A total of 10 000 yeast cell events were acquired, and data were analyzed using CellQuest software (Becton-Dickinson). Viability was expressed as the percentage of live cells (low-fluorescent population on a FSC versus FL1 dotplot) compared to total cells (yeast population on a FSC versus SSC dotplot).

Preparation of Yeast Cells with Alcohol. Yeast was grown at 25 °C for approximately 3 days in YPD with shaking, after which cells were harvested by centrifugation (3000 rpm for 5 min), washed twice with distilled water, and suspended in distilled water. Alcohol was then added to a final concentration of 20−70% (v/v). After alcohol treatment, cells were centrifuged at 2000 rpm for 5 min or filtered using 0.2 μm filter paper. Wet alcohol-treated yeast (ATY) cells were dried at various temperatures in a drying oven (Sanyo, MOV-212). A standard curve relating dry cell weight (g/L) to OD_{600} was generated by suspending a known mass of dried and alcohol-treated cells in distilled water and measuring OD_{600} values of known dilutions. An OD_{600} = 1 was found to be equivalent to 0.89 g of dry cell weight/L.

Treatment of Wine and Grape Juice with ATY Cells. ATY cells were added to wine and juices at various concentrations, and the mixture was shaken sufficiently to disperse the ATY cells. The mixture was kept at room temperature for 1 day, unless otherwise stated. The ATY cells were removed from the treated wines and grape juice by centrifugation at 3000 rpm for 5 min.

Phytic acid [50% (v/v), Wako Chemical] treatment was conducted at 25 °C. After the addition of phytic acid (added to a final concentration), wines or juices were mixed well, and kept for 1 day, and centrifuged at 3000 rpm for 5 min to separate from phytic acid.

The efficacy of iron removal from wine and grape juice using the ATY cells was evaluated by measuring residual iron and comparing the values to concentrations before treatment.

Sensory Analysis. The intensity of fishy aftertaste was assessed by sensory analysis, as described.[8] The sensory panels were comprised of 11 laboratory staff members, 7 men and 4 women ranging in age from 26 to 59 years. All had wine-tasting experience.

Triangle Difference Test. A triangle test was conducted on wine aroma and flavor by comparing an untreated control wine to wine treated with ATY cells followed by a 30 min incubation, centrifugation, and clarification using fining agents. Wine (described above) was treated by the addition of ATY cells at 1, 2, and 3 g/L. Each panelist was presented with three coded glasses of wine and told that one differed from the other two. Panelists evaluated 20 sets of wines in two sessions. Wines treated with ATY cells were always compared to an untreated control.

Effect of Treatment with ATY Cells. The wine treatment with ATY cells was evaluated by its effect on the perceived formation of unpleasant fishy aftertaste, the focus of the present study. Panelists were first given a reference solution, 3 mM $FeSO_4$, as a standard for metallic sensation, a potent factor associated with unpleasant fishy aftertaste, in training sessions to establish approximately equal perceived sensory intensities. Perceived intensity was assessed using the labeled magnitude scales (barely detectable, 1.4; weak, 6.1; moderate, 17.2; strong, 35.4; very strong, 53.3; and strongest imaginable, 100).[7,27] An "other" category was provided for any sensation beyond the unpleasant fishy aftertaste scale. Data are expressed as means of the logarithms of the labeled magnitude scale values.

The commercial dried scallops were used to evaluate wine−seafood pairings with repeated tastings, because dried scallops tend to elicit a strong fishy aftertaste. Evaluations were performed using a sequential dried scallop−wine tasting protocol consisting of chewing and swallowing the dried scallops, taking a sip of wine, and then evaluating the intensity of fishy aftertaste. Drinking water was provided to cleanse

the palate between each dried scallop−wine pairing but not within a dried scallop−wine pairing.

The wine samples were presented in randomly coded glasses. First, panelists tasted wines only. Then, panelists evaluated three wines with dried scallops presented in random order in one session. The wine without ATY cell treatment was used as a positive control.

Measurement of Organic Acid, Alcohols, Esters, and Aldehyde. Organic acids were measured using a high-performance liquid chromatography system (LC-10A, Shimadzu) equipped with an anion-exclusion column Shim-pack SPR-H (250 × 7.8 mm, two-column serial connection), with a mobile phase of 5 mM *p*-toluenesulfonate and a flow rate of 0.8 mL/min at 40 °C. The mobile phase that passed through the column was mixed with a pH-buffered solution [20 mM Bis-Tris, 5 mM *p*-toluenesulfonate, and 80 μM ethylenediaminetetraacetic acid (EDTA)], with subsequent flow at the same rate. The concentration of organic anions was determined using an electroconductivity detector (CDD-10AVP, Shimadzu).

Alcohols, esters, and aldehydes were measured using a gas chromatography system GC-2010 plus (Shimadzu) with a flame ionization detector and a Turbo matrix 40 headspace autosampler (Perkin-Elmer). A DB-1 fused silica column was used (Agilent J&W, Agilent Technologies, Santa Clara, CA), 0.32 mm × 30 m and 5.0 μm film thickness. The injector temperature was maintained at 200 °C with a flame ionization detector (FID) temperature of 200 °C. Helium was used as the carrier at a rate of 25 cm/s. The oven temperature was held at 40 °C for 10 min. The column was programmed from 40 (3 min) to 90 °C at 4 °C/min and from 90 to 180 °C (2 min) at 20 °C/min.

Measurement of Iron. Iron in wine and grape juice was determined by atomic adsorption spectrometry (Analytik Jena, contrAA700) in an air/acetylene oxidizing flame. Fe was detected at 248.327 nm. Samples were diluted 3−10 times with deionized ultrapure water to avoid matrix effects. Results were recorded as milligrams per liter to two significant digits.

Data Analysis. Statistical analysis was performed using Excel 2000 (Microsoft, Redmond, WA) with the add-in software Statcel 2.[28] The effect of co-treatment of phytic acid and ATY cells on iron removal was analyzed by Student's *t* test. The effect of order of addition of phytic acid and ATY cells on iron removal was analyzed by one-way analysis of variation (ANOVA). The effect of phytic acid and ATY cells on the intensity of fishy aftertaste was analyzed by two-way ANOVA, followed by Tukey's post-hoc tests. The triangle difference test was analyzed by the calculation of *p* values. Differences were considered significant when the *p* value was less than 0.05.

RESULTS AND DISCUSSION

Investigation of Yeast Iron Uptake during Wine Fermentation. To investigate changes in the iron concentration during wine fermentation, we carried out test fermentations using various wine yeast strains. In general, fermentations were complete after 7 days at 20 °C, by which time sugar had been completely consumed. Figure 1 is a representative example showing grape juice fermentation conducted using wine strain OC-2. Precultured wine yeast cells adsorbed iron rapidly at the early stage of fermentation from 0 to 10 h (Figure 1a). Gradual release of iron was observed after 10 h of incubation (Figure 1a). Relative to previous observations, the OC-2 wine strain did not take up iron during fermentation as well as a laboratory strain of *Saccharomyces cerevisiae* that did not take up much iron when grown anaerobically.[29] However, at the post-fermentation stage (more than 100 h of incubation), as the proportion of dead cells rapidly increased, the concentration of iron in wine gradually decreased (Figure 1b).

Development of Ethanol-Treated Yeast (ETY) Cells as an Iron Adsorbent. On the basis of the results shown in Figure 1, we hypothesized that dead yeast cells had a significant capacity to adsorb iron from wine. To test this hypothesis, cells were killed by either heat treatment (65 °C) or treatment with ethanol.

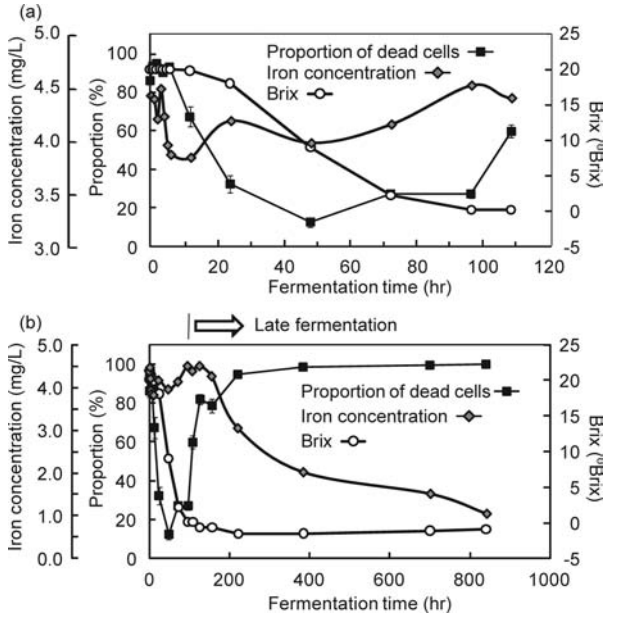

Figure 1. Fermentation profile of wine yeast OC-2 during static cultivation in white grape juice supplemented with diammonium phosphate. (a) Early fermentation phase until sugar depletion. (b) Complete fermentation time course, including 840 h of post-fermentation. Data are means of three independent experiments (±standard deviation).

In contrast to the heat-treated cells that were found unable to adsorb iron, the ethanol-killed cells retained a significant adsorbent ability (Figure 2). This is consistent with what was observed

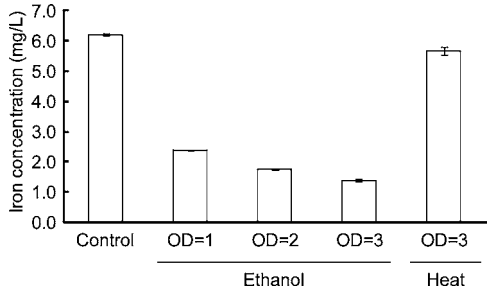

Figure 2. Amount of iron removed as a function of added ETY cells. Wine yeast DV10 cells were treated with 70% (v/v) ethanol for 12 h or heated at 65 °C for 1 h, respectively. The treated yeast cells were added to grape juice and held for 1 day. The amount of yeast cells is indicated in OD_{600} units (OD). Data are means of three independent experiments (±standard deviation).

during fermentation. After about 100 h, almost all of the cells were dead, while iron levels dropped continuously (Figure 1b).

It has been reported that pairing seafood with wine containing approximately ≥1 mg/L iron will result in perceived fishy aftertaste.[8] ETY cells were found to reduce iron concentrations in grape juice from 6.2 to 1.3 mg/L when added at a concentration equivalent to OD_{600} = 3. The enhanced iron-adsorbing ability of *S. cerevisiae* and *Saccharomyces pastorianus* by ethanol treatment was confirmed in laboratory, wine, and beer strains (Table S1 of the Supporting Information).

Optimization of Conditions To Treat Yeast Cells with Ethanol. To optimize conditions to treat cells with ethanol, the ethanol concentration and duration of treatment were investigated. Regardless of the concentration, short treatment

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times, <30 min, were found to be most effective (Figure 3). The total mass of ETY was found to decrease as the period of ethanol

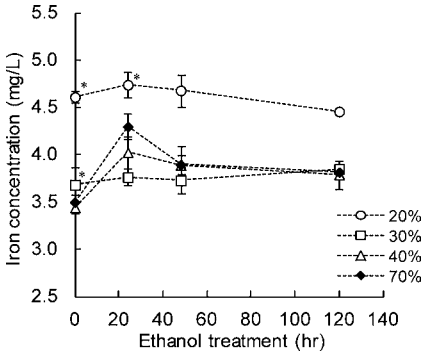

Figure 3. Iron adsorption ability of yeast DV10 cells as a function of the ethanol concentration and treatment time. The ethanol treatment was performed at 37 °C. Cells were added to juice at OD_{600} = 1 and held for 1 day. The initial iron concentration in grape juice was 6.2 mg/L. (*) ETY cells were not all nonviable. Data are means of three independent experiments (±standard deviation).

treatment increased. For example, of 95 mg of yeast mass treated with 70% (v/v) ethanol, 95, 83, and 64 mg were recovered after 30 min, 1 day, and 3 days, respectively. We found that >40% (v/v) ethanol was necessary for effective iron adsorption presumably because a fraction of the treated cells remained viable following treatment with ≤30% (v/v) ethanol (Figure 3). Iron removal treatments of wine using cells treated with <30% (v/v) ethanol should be avoided to prevent possible refermentation and associated complications.

The next step in optimization was to determine if the temperature used to dry ETY cells affected the efficacy of iron removal. The ETY cells used in the experiments depicted in Figures 2 and 3 were not dried. As shown in Figure 4, once

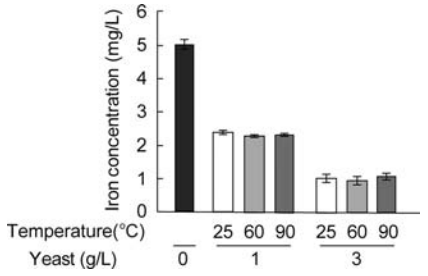

Figure 4. Effect of the temperature used to dry ETY cells on iron adsorption. Ethanol-treated DV10 cells were added to juice at 1 or 3 g/L and held for 1 day. The initial iron concentration in grape juice was 5.0 mg/L. Data are means of three independent experiments (±standard deviation). No significant differences among the temperatures used were observed (one-way ANOVA; $p < 0.05$).

yeast cells were treated with ethanol, iron adsorption capacity was not affected by the drying temperature within the range of 25–90 °C.

The efficacy of iron adsorption by ETY cells was tested after storing the dried cells for 1 month. Cells that had been treated with 70% (v/v) ethanol and then dried were used. The 1 month of storage did not reduce the efficacy of iron adsorption. Regardless of whether 1-month-old or newly dried cells were used, 47 and 72% of the iron present in grape juice was removed by use of 1 and 3 g/L cells, respectively (Figure 5). Iron was

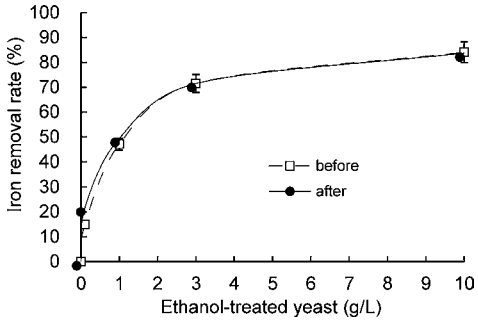

Figure 5. Iron adsorption by ETY cells before and after 1 month of storage at room temperature. Ethanol-treated DV10 cells were added to juice and held for 1 day. Initial and final iron concentrations were 6.4 mg/L (before) and 5.4 mg/L (after), respectively. The iron removal rate (%) was calculated on the basis of the amount of iron remaining after treatment with the ETY cells. Data are means of three independent experiments (±standard deviation).

reduced to about 1.4 mg/L from an initial concentration of 5 mg/L by the addition of 3 g/L ETY cells.

Advantages of ETY Cells as Iron Adsorbents in Wine. We compared the iron-removing ability of ETY cells to phytic acid, which is generally used as an iron adsorbent in beverage and alcohol production.[22,23] When iron binds to phytic acid, the resulting iron–phytic acid complex precipitates. However, the efficacy of phytic acid is, in part, dependent upon matrix effects and the presence of competing cations. In some cases, we have observed little iron removal from wine or concentrated grape juice by phytic acid (data not shown). The addition of ETY cells to wine pretreated with phytic acid was found to remove the residual iron left behind (Figure 6). A synergistic effect on

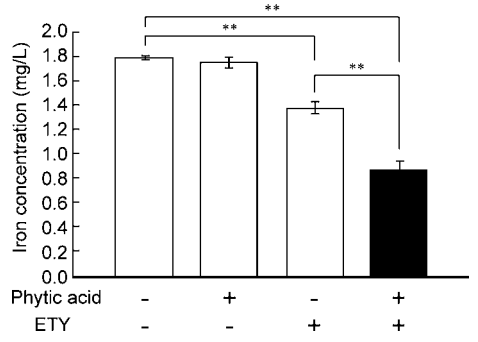

Figure 6. Co-treatment of white wine (wine A) with phytic acid and ETY cells (Dia yeast) removes iron in a synergistic manner. White wine was treated with phytic acid and ETY cells at 20 mg/L and 3 g/L, respectively, and held for 1 day. Data are means of three independent experiments (±standard deviation). (**) Significant differences ($p < 0.001$) between the indicated pairs of treatments.

iron removal was observed when white wine was treated with a mixture of ETY cells and phytic acid (Figure 6). This effect was also observed when as little as 0.5 g/L ETY cells was added.

With respect to the order of addition of ETY cells and phytic acid, the addition of ETY cells following phytic acid addition was found to be the most effective (black bar in Figure 7). We presume that pre-addition of phytic acid led to greater adsorption because of the fact that formation of the insoluble phytic acid–iron complex that is removable by centrifugation or filtration did

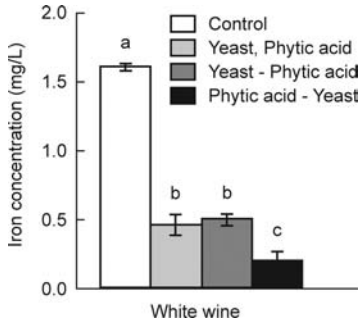

Figure 7. Effect of the order of addition of phytic acid and ETY cells (Dia yeast) in wine (wine A): white bar, wine without ETY cell treatment (control); light gray bar, co-addition of ETY cells (3 g/L) and phytic acid (20 mg/L); dark gray bar, ETY cells added first and incubated for 1 day, followed by phytic acid addition; and black bar, phytic acid added first and incubated for 1 day, followed by the addition of ETY cells. Data are means of three independent experiments (±standard deviation). Values with different letters (a, b, and c) are significantly different at $p < 0.05$ by one-way ANOVA.

not occur immediately. Thus, residual-free soluble phytic acid still present upon the addition of ETY cells retained iron-binding ability.

Evaluation of ATY Cells as Iron Adsorbents. Because of the expense and legal complications associated with use of large volumes of high-proof beverage alcohol, we evaluated alcohols other than ethanol for their ability to increase the iron-adsorbing ability of yeast. Table 3 shows that all of the tested alcohols, including *n*-propanol and isopropyl alcohol, were also effective.

Table 3. Iron Adsorption by Various ATY Cells[a]

alcohol	iron concentration (mg/L)
ethanol	3.36 ± 0.07
n-propanol	4.16 ± 0.14
isopropyl alcohol	4.06 ± 0.06
n-butanol	3.66 ± 0.03
isoamyl alcohol	3.74 ± 0.10

[a]SYT001 cells were treated with 70% (v/v) of each alcohol. The dried alcohol-treated SYT001 were added to juice at 3 g/L and incubated at 25 °C for 1 day. The initial iron concentration of juice was 6.5 mg/L. Data are means of three independent experiments (±standard deviation).

Sensory Evaluation of Fishy Aftertaste in Wines Treated for Iron Removal. Previous work has shown that ferrous ion is a key compound in the formation of fishy aftertaste in wine and seafood pairing.[8] In the present study, we tested methods for removing iron from wine (and grape juice) and subjected the treated wines to sensory evaluation.

First, we investigated whether the character of wine was changed by the addition of ATY cells using the triangle difference test. Panelists were able to differentiate the control wine and wine treated with ETY cells at 3 g/L but not at 1 or 2 g/L (Table 4). Panelists did not describe the difference as an increase of flavor, including yeasty character, suggesting that the addition of the ETY cells at ≥3 g/L may have resulted in a change of wine constituents.

Second, white wine was treated with isopropyl alcohol-treated yeast (ITY) cells and phytic acid and then evaluated for fishy aftertaste using dried scallops, as described in the Materials and Methods. Wine treated with ITY cells had less fishy aftertaste than wine treated with phytic acid alone (Figure 8). Wine treated with both ITY cells and phytic acid produced the least fishy aftertaste (Figure 8). A two-way ANOVA indicated no major effect of panelists [$F(10, 30) = 1.97$; $p = 0.073$] but a significant major effect of samples [$F(3, 30) = 21.1$; $p = 1.48 \times 10^{-7}$] on the intensity of fishy aftertaste. Although Table 4 suggests that ETY cell addition at 3 g/L led to a change of wine character, no significant sensation was indicated in the "other" category scale. We speculate that the sensory differences between wines treated with and without 3 g/L ETY cells assessed in seafood pairings were not great enough to be discernible as specific sensory defects by the panelists.

Table 4. Triangle Difference Test of Aroma and Flavor Comparing an Untreated Control Wine to Wines Treated with ETY Cells

ETY cells (g/L)[a]	correct response	total response	*p* value
1	10	20	0.054
2	7	20	0.182
3	11	20	0.024

[a]Ethanol-treated IFO1167 strain.

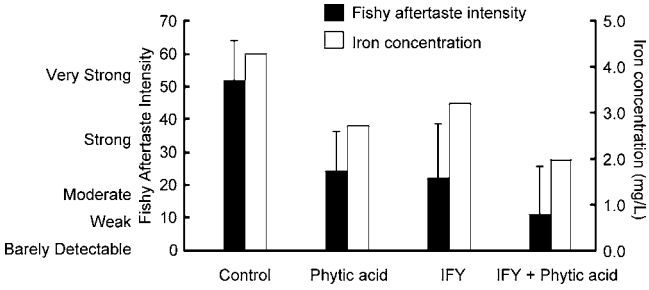

Figure 8. Effect of ITY (Dia yeast) cells and phytic acid on the intensity of fishy aftertaste in white wine paired with dried scallop. Dried ITY cells were added at 3 g/L, and phytic acid was added at 50 mg/L, followed by a 30 min incubation. Before tasting, each treated white wine was clarified by the addition of fining agents. Data are means of 11 replicates (±standard deviation).

Finally, to determine whether other major chemical constituents in wine relevant to sensory quality might have been removed by the treatment, we measured the concentration of organic acids (formic acid, acetic acid, succinic acid, malic acid, lactic acid, citric acid, pyruvic acid, phosphoric acid, and pyroglutamic acid), esters (ethyl acetate and isoamyl acetate), alcohols (1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and isobutanol), and an aldehyde (acetaldehyde). No differences were observed in the concentrations of these constituents between untreated control white and red wines and their treated counterparts (Tables S2 and S3 of the Supporting Information).

General Discussion. We developed a method to enhance the iron-adsorbing ability of yeast by treatment with ≥40% (v/v) ethanol. Drying of the ATY cells was not found to reduce iron-adsorbing ability. A correlation between iron removed from wine and grape juice and the amount of ATY cells added was established.

Phytic acid has been used in the food industry as a safe and stable compound to remove iron. However, we found that phytic acid is not effective in all wines and juices. ATY cells may find application for such "problematic" wines and juices.

One practical problem associated with the use of ATY cells has to do with the cost of filtering a dense yeast suspension. To reduce the potential of a grape juice containing 5 mg/L iron from producing a wine prone to fishy aftertaste in seafood pairings, we found it necessary to use 3 g/L ATY cells. Subsequent filtration of juice containing this high amount of suspended cells was time-consuming. The finding that the use of phytic acid in combination with ATY cells ("ATY−PA method") yielded a synergistic increase in iron removal suggests a way to reduce the amount of yeast needed.

Iron in wine has sometimes been recognized as a cause of the decrease of wine quality. For example, at relatively high concentrations, ∼10 mg/L, iron can induce the formation of precipitates (casse). At lower levels (a few milligrams per liter), in addition to causing the fishy aftertaste, iron promotes the formation of hydroxyl radicals by the Fenton reaction and catalyzes the oxidation of phenolic compounds that can change the chemical and sensory profile of wines.[30] The ATY−PA method may allow for the resolution of these potential problems.

More work is needed to better characterize ATY cells and to better understand how and why they bind iron. Identification of the iron-binding components is likely to lead to the development of a better adsorbent.

■ ASSOCIATED CONTENT

Supporting Information

Iron adsorption ability of ETY strains (Table S1), change in organic acid concentrations following the addition of ETY cells (Table S2), and changes in concentrations of selected volatile constituents following the addition of ETY cells (Table S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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