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Research Article

Effect of additives on the fermentative ability of commercial dry yeast in the production of mead

Ubukata Shuichiro[1]✉, Seki Hiroko[1]

[1]*School of Bioscience and Biotechnology, Tokyo University of Technology, Tokyo, Japan*

Abstract

Honey contains many monosaccharides as fermentation materials for mead production, but little nitrogen or minerals. Consequently, the quality of the final product is inconsistent owing to a lack of nutrients. Here, we sought to identify valuable additives that can be used as nutrient sources for efficient mead production. We examined the effects of diammonium hydrogen phosphate, ammonium sulfate, and salts on the alcoholic fermentation of honey must by dry yeast. The results showed that the alcohol concentration in the mead increased with the addition of diammonium hydrogen phosphate but decreased with the addition of ammonium sulfate. The addition of salts such as potassium chloride, potassium dihydrogen phosphate, magnesium chloride, magnesium sulfate, or sodium chloride increased the alcohol concentration, whereas the addition of sodium carbonate or calcium chloride inhibited fermentation. Our findings indicate that the addition of diammonium hydrogen phosphate, potassium chloride, potassium dihydrogen phosphate, magnesium chloride, magnesium sulfate, and sodium chloride is effective in the production of mead using. the dry yeast employed in this study.

Keywords
honey, mead, yeast, fermentative ability, alcoholic fermentation

✉*Corresponding author: Seki Hiroko, School of Bioscience and Biotechnology, Tokyo University of Technology, Tokyo, Japan, tel.: +81-42-637-2193; E-mail: sekihrk@stf.teu.ac.jp*

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Introduction

Mead is a brew produced from honey, and the alcoholic fermentation during its production uses the glucose and fructose present in honey (Pereira et al. 2015). Alcoholic fermentation has the greatest impact on the quality of the mead produced; however, owing to the lack of detailed reports on yeast fermentation conditions, the quality of the final product is not consistent (Iglesias et al. 2014). Furthermore, problems such as midway cessation of alcoholic fermentation and undesirable taste and flavor production are associated with mead production (Pereira et al. 2009). These problems are related to the inability of yeast strains to adapt to stressful environments (Pereira et al. 2009), including the lack of nutrients for yeast growth.

Honey is mostly composed of sugars and contains very little nutrient content (U.S. Department of Agriculture 2022). Therefore, nitrogen compounds must be added as nutrient sources during its fermentation. Supplementation of wine must with diammonium hydrogen phosphate during fermentation enhances the growth of *Saccharomyces cerevisiae* PYCC 4072, fermentation rate, and ethanol value (v/v) (Mendes-Ferreira et al. 2004). In contrast, the addition of 0.5 $g.L^{-1}$ N as ammonium sulfate to cassava medium prepared for alcoholic fermentation decreases the fermentative ability of *S. cerevisiae* TG1348 owing to a significant decrease in the pH of the medium during the fermentation process (Yang et al. 2022). These findings indicate that different additives have different effects on yeast fermentation.

The metal ions present in the must during fermentation affect the fermentation potential of the yeast; thus, potassium is involved in osmotic pressure and alcohol tolerance in yeast (Xu et al. 2019). Magnesium, also a metal ion, is an essential cofactor in cell metabolism (Ribeiro-Filho et al. 2021). Ethanol production by *S. cerevisiae* NCYC2592 increases with the addition of potassium and magnesium, whereas that by *Saccharomyces pastorianus* W34/70 increases slightly with the addition of magnesium but decreases with the addition of potassium (Ribeiro-Filho et al. 2021). In addition, when the effect of calcium chloride on the growth of the yeast present in pepper mash was investigated, a decrease or increase in the number of viable yeasts was reported, depending on the number of days of storage (Flores et al. 2007). These findings suggest that different metal ions have different effects on yeast fermentation.

Therefore, the present study aimed to examine the effects of different nutrients and salts on the fermentative potential of dry yeast for mead production. To the best of our knowledge, this is the first study to investigate the effects of different concentrations of diammonium hydrogen phosphate, ammonium, sulfate, and various types of metal ions on the fermentative potential of yeast in mead production. We hypothesized that varying concentrations of diammonium hydrogen phosphate, ammonium sulfate, and different metal ions would significantly affect the fermentative potential of dry yeast.

Materials and Methods

Materials. Super Camellia dry yeast granules obtained from Nisshin Seifun Welna Inc. (Tokyo, Japan) designed for home bakery, was purchased from a retail store in Hachioji City and used as the dry yeast in the experiments. Acacia honey obtained from Ou Apiary (Akita, Japan) was used in this study.

Effect of nitrogen compounds on mead production. First, 0.2 g dry yeast was added to 210 g distilled water and mixed. The dry yeast granules contain emulsifiers, yeast extract, and vitamin C. Emulsifiers were used in the fermentation process, and fermentation tests were conducted using distilled water to achieve a certain Brix value (Mukai et al. 2013). In the present study, distilled water was also used to dissolve the yeast. Subsequently, diammonium hydrogen phosphate or ammonium sulfate was added to yield a final concentration of 0, 30, 90, 150, 210, and 270 mg. 300 ml^{-1}. Then, 90 g honey was added to the prepared solution and mixed to prepare 300 g must, with a honey concentration of 30% w/w. The amount of yeast added to the must was determined based on a study by Czabaj et al. (2017). After alcoholic fermentation for one week in a thermostatic oven set at 30℃, 200 ml of mead was transferred to a simple distiller and distilled on a household induction cooktop set at 90℃ until the distillate volume reached 100 ml. The distillate was cooled overnight in an incubator set at 15℃ and

then mixed with 200 ml of distilled water (15°C). The alcohol content of the mixture was measured using an alcohol meter (KSS-01, Ando Keiki Co., Ltd., Tokyo, Japan). The method used to measure alcohol concentration met the standard value for recovery rate under Japanese law (National Tax Agency prescribed analysis method 1961).

Time-dependent changes in the pH of the must with the addition of ammonium sulfate. The pH of the musts described above was measured using a benchtop pH meter (F-71, SS112, Laqua Horiba Ltd., Kyoto, Japan) every 24 h throughout the fermentation period, from 0 h (when the musts were prepared) to completion at day 7.

Effect of salts on mead production. The must was supplemented with each of the salts listed in Table 1. The fermentation conditions and alcohol concentration measurements were as described above.

Table 1. Amounts of the salts added

Salt	Amount added, g	Reference
Potassium chloride	3.00	Xu et al. (2020)
Potassium dihydrogen phosphate	5.48	Original[1]
Magnesium chloride hexahydrate	3.04	Original[2]
Magnesium sulfate heptahydrate	3.69	Sharma et al. (2021)
Sodium carbonate	3.99	Original[1]
Sodium chloride	3.62	Original[1]
Calcium chloride	1.66	Original[2]

*The amounts were determined according to Sharma et al. (2021), and Xu et al. (2020)

**Original concentrations were prepared by matching the molar concentrations with the values used in the corresponding studies: [1]:Xu, [2]:Sharma

Measurements of the pH of must with each salt. Honey (90 g) was dissolved in 210 ml distilled water. Various salts were added to the solution according to Table 1. The pH of the solution was measured using a benchtop pH meter (F-71, SS112, Laqua Horiba Ltd., Japan).

Effect of calcium chloride on yeast fermentation. A 300 ml glucose solution with a concentration of 30% w/w was prepared by mixing 90 g glucose with 210 ml distilled water. Then, 1.66 g calcium chloride was added to the solution (final concentration: 5.55% × 10^{-3} w/w). The prepared glucose and calcium chloride solution (30 ml) and yeast (1 g) were placed in a Kühne fermentation tube and thoroughly stirred. The tube was allowed to stand for 30 min, and the amount of gas produced was measured.

Statistical analysis. Data were obtained in triplicate, adhering to Fisher's three principles. Biological replicates were used in this experiment. A two-tailed test was conducted, and differences in means were assessed using unpaired *t*-tests. Comparisons among three or more conditions were made using one-way analysis of variance. The significance level was set at 5% for all experiments. All the tests were performed using Microsoft Excel (Microsoft Office Home and Business 2019, Microsoft Corporation, Redmond, WA, USA).

Results and Discussion

Table 2 shows the alcohol concentration in each of the musts fermented with different concentrations of diammonium hydrogen phosphate. The concentration increased from 2.13% at a final diammonium hydrogen phosphate concentration of 0 mg.300 ml^{-1} to 5.13% at 270 mg.300 ml^{-1} ($p < 0.05$). Thus, there was a substantial increase in the fermentative capacity of the dry yeast with increasing concentrations of diammonium hydrogen phosphate. This trend is similar to that observed in a previous study examining fermentation by *Saccharomyces cerevisiae* AWRI 796, in which the addition of 150 and 300 $mg.l^{-1}$ diammonium hydrogen phosphate to wine shortened the time to reach the target alcohol concentration by four days (Ugliano et al. 2008). However, no differences in fermentation rate or duration were observed when 250 $mg.l^{-1}$ N diammonium hydrogen phosphate was added to

wine must containing *Saccharomyces cerevisiae* M05 (Vilanova et al. 2012). These differences suggest that the same nitrogen source may have different effects on fermentation, depending on the type of yeast.

Table 3 shows the alcohol concentration in musts fermented with different concentrations of ammonium sulfate. The concentration decreased from 2.83% to 0.53% as the ammonium sulfate concentration decreased from 0 mg.300 ml^{-1} to 270 mg.300 ml^{-1} ($p < 0.05$).

Table 2. Alcohol concentration in musts fermented at different diammonium hydrogen phosphate concentrations

Diammonium hydrogen phosphate, mg.300 ml^{-1}	**Average alcohol concentration, % v/v**
0	2.13±0.06
30	1.90±0.40
90	2.03±0.25
150	2.77±0.06
210	4.43±0.49
270	5.13±0.35

Data obtained from triplicate samples. All data from 0 mg.300 ml^{-1} to 270 mg.300 ml^{-1} are significantly different as per analysis of variance ($p < 0.05$)

Thus, there was a significant decrease in the fermentative capacity as ammonium sulfate increased. This corresponds with the results of a previous study where the alcoholic fermentation of cassava medium by *Saccharomyces cerevisiae.*

Table 4 shows the pH variations of the mead over time following the addition of different concentrations of ammonium sulfate.

The faster rate of decrease in pH with increasing ammonium sulfate concentration (0 h: $p > 0.05$, 24–168 h: $p < 0.05$) suggests that the fermentation capacity is decreasing correspondingly. Notably, the pH decreased over time regardless of the concentration of ammonium sulfate ($p < 0.05$), suggesting that there is a relationship between the decrease in pH and alcohol concentration of ammonium sulfate (Table 4) suggesting that there is a relationship between the decrease in pH and alcohol concentration.

TG1348 in the presence of 0.5 $g.L^{-1}$ N as ammonium sulfate was shown to be inhibited (Yang et al. 2022).

Table 3. Alcohol concentration in musts fermented with different ammonium sulfate concentrations

Ammonium sulfate, mg.300 ml^{-1}	**Average alcohol concentration, % v/v**
0	2.83±0.32
30	2.70±0.17
90	1.90±0.40
150	1.53±0.21
210	1.30±0.26
270	0.53±0.29

Data obtained from triplicate samples. All data from 0 mg.300 ml^{1} to 270 mg.300 ml^{-1} are significantly different as per analysis of variance ($p < 0.05$)

Table 5 shows the alcohol concentration in musts fermented with different salts. The alcohol concentrations in the meads produced in the presence of potassium chloride, potassium dihydrogen phosphate, magnesium chloride, magnesium sulfate, and sodium chloride were higher than that in the control ($p < 0.05$). In contrast, the addition of sodium carbonate resulted in alcohol concentrations (2.60%) were slightly lower than that in the control ($p > 0.05$). However, only the addition of calcium chloride resulted in a significantly lower alcohol concentration than that in the control ($p < 0.05$). The inorganic nutrients available to yeasts differ among yeast species and strains. The addition of potassium and magnesium reportedly increases ethanol production by *Saccharomyces cerevisiae* NCYC2592 (Ribeiro-Filho et al. 2021).

Similarly, the dry yeast used in this experiment showed an increase in fermentative capacity with the addition of potassium and magnesium. Table 6 lists the pH values of the aqueous salt solutions. Most of the solutions were mildly acidic, with their pH ranging from 3 to 4. In contrast, the sodium carbonate solution was strongly basic (pH = 10.53). The alcohol concentration in mead produced with the addition of sodium carbonate was slightly lower than that in the control (2.60% versus 2.83% v/v).

Table 4. Time-dependent changes in the pH of meads produced with different concentrations of ammonium sulfate

Ammonium sulfate, g.300 ml^{-1}	pH of mead during alcoholic fermentation								
	0 h	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**	**144 h**	**168 h**	**p**
0	3.84 ±0.04	3.61 ±0.02	3.24 ±0.02	3.09 ±0.04	3.05 ±0.02	2.90 ±0.04	2.84 ±0.05	2.74 ±0.04	*
30	3.81 ±0.01	3.42 ±0.08	2.92 ±0.03	2.81 ±0.05	2.87 ±0.02	2.84 ±0.03	2.80 ±0.02	2.64 ±0.03	*
90	3.81 ±0.01	2.87 ±0.05	2.58 ±0.01	2.65 ±0.25	2.62 ±0.00	2.62 ±0.01	2.56 ±0.05	2.38 ±0.13	*
270	3.81 ±0.01	2.77 ±0.01	2.60 ±0.01	2.51 ±0.01	2.64 ±0.01	2.64 ±0.01	2.62 ±0.01	2.37 ±0.10	*
p	**	*	*	*	*	*	*	*	

Data obtained from triplicate samples.
* Significantly different values as per analysis of variance ($p < 0.05$).
** Not significant values as per analysis of variance ($p > 0.05$).

Table 5. Alcohol concentration in meads produced with different salts

Additive	Alcohol concentration, % v/v
No additive (control)	2.83±0.32
Potassium chloride	4.37±0.38*
Potassium dihydrogen phosphate	4.47±0.40*
Magnesium chloride	4.60±0.26*
Magnesium sulfate	5.27±0.15*
Sodium carbonate	2.60±0.20**
Sodium chloride	4.47±0.06*
Calcium chloride	1.23±0.15*

Data obtained from triplicate samples. * Significantly different values as per analysis of variance ($p < 0.05$).
** Not significant values as per analysis of variance ($p > 0.05$).

Saccharomyces cerevisiae shows optimal growth in the fermentation medium at pH 3.5-5.0 (Buzás et al. 1989). Therefore, the fermentative ability of the dry yeast in the must containing sodium carbonate may have decreased because the pH of the must deviated substantially from the optimal pH. The pH of the no-additive (control) was pH 3.78.

Table 6. pH of the must with each salt

Additive	Amount added, g	pH
Potassium chloride	0.50	3.65
Potassium dihydrogen phosphate	0.91	4.04
Magnesium chloride	0.51	3.54
Magnesium sulfate	0.62	3.66
Sodium carbonate	0.67	10.53
Sodium chloride	0.60	3.93
Calcium chloride	0.28	3.42

The addition of $CaCl_2$ resulted in a decrease in the alcohol concentration (Table 5). Therefore, the inhibitory effects of Ca on the fermentative potential of yeast was tested using glucose solution instead of honey. When a 30% glucose solution was fermented with $CaCl_2$ added to reach a final concentration of 5.55×10^{-3}% (w/w), the average gas production (ml) was 2.31 ± 0.58 ml (n = 3) compared to 3.98 ± 1.40 ml (n = 3) without calcium chloride addition.

In a previous study that investigated the effects of calcium chloride on yeast growth in pepper mash (Flores et al. 2007), the researchers added 8 g.100 g^{-1} $CaCl_2$; at the end of day 1, the viable count in the control was 12.45 ln CFU.g^{-1}, whereas that with the addition of calcium chloride was 9 ln CFU.g^{-1}. However, on day 24, the viable counts were 13.75 and 10.27 ln CFU.g^{-1} in the mashes with and without $CaCl_2$, respectively. These results suggest that the yeast flora may have changed over time, with an increase in the proportion of cells that could effectively use calcium chloride, thereby limiting the growth of cells that could not thrive in such conditions and reducing their proportion of the total population by day 24. The dry yeast used in this study was not considered resistant to $CaCl_2$ because the addition of $CaCl_2$ reduced the amount of gas produced.

Notably, the present study had a few limitations. For example, all components present in honey were not elucidated; moreover, honey samples used for the study did not always have consistent compositions. Nonetheless, the findings of the study suggest the importance of considering nutrient additives during the fermentation process, which can help optimize mead production.

Conclusions

The present study examined the effects of diammonium hydrogen phosphate, ammonium sulfate, and different salts on alcoholic fermentation by dry yeast during mead production. The addition of diammonium hydrogen phosphate, potassium chloride, potassium dihydrogen phosphate, magnesium chloride, magnesium sulfate, and sodium chloride increased the fermentative capacity and alcohol concentration, indicating that the addition of these ingredients is effective in the production of mead with dry yeast. Since it was found that mead can be efficiently produced under these conditions, addition of these salts can be said to be practicable for mead production. These results can contribute towards improving the production of mead and the brewing industry as a whole. Mead is affected by the quality of honey, and as this is influenced by harvest time, region, and other factors, future work is required to establish the benefit of the addition of ammonium salts and metal ions to fermentations involving honey of different qualities.

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