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The Delay of Ginseng Wine Fermentation: The Effects of Ginseng Extrusion Temperature, Sugar Source, Fermentation Temperature, and Diammonium Phosphate on the Fermentation

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Abstract To overcome the problem of ginseng's earthy smell in the manufacture of ginseng wine, we used dried ginseng powder that was extrusion-cooked at 125-168°C in the wine making process. By using a ginseng powder that was extrusion-cooked at higher temperatures, fermentation by Maillard reaction products (MRPs) was delayed, and the acidic pH that results from extrusion cooking was improved. At 15°C with glucose instead of sucrose, an addition of 0.5%(w/v) diammonium phosphate (DAP) to the 125°C extrusion-cooked ginseng powder reduced the primary fermentation time to 11 days versus 33 days without DAP. In the absence of DAP, by increasing the fermentation temperature from 15 to 30°C, increasing the starter yeast inoculate from 0.02 to 1%, and by increasing the amount of ginseng extrudate from 1 to 2%, fermentation time was effectively reduced more than 10-fold. The results of this study may provide information for the alcohol fermentation of materials containing MRPs as well as for poor nitrogen sources.

Keywords: extrusion, ginseng wine, fermentation delay, yeast

Introduction

Panax ginseng C.A. Meyer has been used as a pharmacological material for a long time in Eastern Asia in terms of its antitumor, antistress, antineoplastic, antioxidant, antiaging, and immune system improving effects (1-4). Ginseng products such as ginseng teas, liquid ginseng extracts, ginseng capsules, ginseng drinks, ginseng wines, etc. have been widely consumed in Korea (5).

In the production of ginseng wine, ginseng's earthy smells as well as fermentation delay are primary obstacles for the ginseng wine industry (6). It is known that extrusion treatments of ginseng can remove its earthy smell and improve flavor. Also, the compounds panacene, β-elemene, and panaxynol are volatilized and degraded, along with the earthy smell, by high temperature popping during the extrusion treatment (7). Earlier, it was reported that ginseng concentrations greater than 6-10% inhibited yeast fermentation, and ginseng extract as well as saponins inhibited *Zymomonas mobilis* growth (6, 8). Moreover, in our previous study, we found that the fermentation time of ginseng wine was longer when the processing temperature was higher (raw ginseng < white ginseng < red ginseng < extrusion-treated ginseng powder). It was also reported that carbohydrate and amino acid mixtures, from Maillard reaction products (MRPs) formed in the thermal process, reduce the alcohol produced during yeast fermentation (9). This delay of fermentation can cause yeast autolysis after exhaustion of the external nutrient supply (10). In addition, we know that fermentation delay during wine production can be attributed to nitrogen deficiency, lack of oxygen, juice clarification, the inhibition of yeast cell activity by fermentation by-products, and a low pH. Because *Saccharomyces cerevisiae* uses rich nitrogen sources over poor nitrogen sources, diammonium phosphate (DAP) is widely used to prevent nitrogen-related problems in production wineries (11). Temperature is one of the most influential factors affecting fermentation, such as by yeast growth and the production of volatile compounds (12). In an attempt to overcome the phenomenon of yeast autolysis, we increased the yeast inoculum to maintain a high yeast number.

The objectives of this study were to manufacture ginseng wine without an earthy smell by using treated ginseng extrudate, and to investigate the factors involved in fermentation delay in ginseng wine, especially when MRPs are formed. These factors included the ginseng extrusion temperature, carbon source, fermentation temperature, ginseng concentration, and the addition of DAP.

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Materials and Methods

Ginseng component and extrusion conditions White ginseng that was cultivated for 4 years in Gumsan, Korea, was dried and milled under 60 mesh. The powder form of the white ginseng (lipid 1.60, protein 15.97, ash 7.34, fiber 6.12, and starch 18.5% d.b.) was extruded by a co-rotating intermeshed type twin-screw extruder (DNDL-44; Buhler Brothers Co., Uzill, Switzerland) in the Korea Food Research Institute, with an L/D ratio of 20:1, as in the method of Jee *et al.* (5). The heating temperature range was 75-180°C. The diameter of the die exit was 2 mm. The feed rate, moisture content, and extruder screw speed were 20 kg/hr, 15-30%, and 3,000 rpm, respectively.

Ginseng wine fermentation The dried ginseng powder extrusion-treated at 125, 135, 140, 150, 160, or 168°C was mixed with commercial *S. cerevisiae* (Fermivin, DMS Food Specialties, Macon, France) and a carbon source in

water, and then incubated under static culture conditions at 15 or 30°C. A 0.02 or 1%(w/v) amount of the dried wine yeast was inoculated to a 1 or 2%(w/v) amount of the ginseng extrudate (0.5-6.0%, w/v) containing a 23°Bx carbon source of sucrose or glucose. Final solution volumes of 1 L that contained the mixture of the carbon source, ginseng, and *S. cerevisiae* were placed in 2-L Pyrex bottles with loosened screw caps, to accommodate expansion by CO_2 production during fermentation. DAP (Sigma, St. Louis, MO, USA) was added, and the mixture was well homogenized before fermentation. When the Brix value reached 7-8°Bx and the alcohol content reached 11-12%, the product was transferred to a 15°C incubator for the second stage of fermentation for 7 days. The ginseng and yeast were removed by pouring and filtering the solution. Meanwhile, ginseng was also added to a potato dextrose broth (Difco Lab., Detroit, MI, USA) with a sucrose solution to compare the consumption of sucrose by the yeast when sufficient nutrients were present.

pH The pH of the ginseng solution was measured using a pH meter model EA 940 (Orion, Boston, MA, USA).

Brix measurement The Brix of the solution was read using a refractometer model H-50 (Atago, Torrance, CA, USA) to monitor the carbon source consumption by the yeast in the first fermentation of the ginseng wine.

Alcohol content The alcohol content produced during wine fermentation was analyzed using a gas chromatographer model Vista 6000 (Varian, Walnut Creek, CA, USA) by loading a filtered 1 μL wine sample. The peak area of the sample was transformed to alcohol content according to the standard curve of ethyl alcohol.

Yeast enumeration The cell number of dried wine yeast during fermentation was determined by plate counting using potato dextrose agar (Difco) and incubation at 30°C for 24 hr. The cell number was expressed as colony-forming units per mL.

Results and Discussion

The effects of extrusion temperature, nutrients, and pH on ginseng wine fermentation To remove ginseng's earthy smell, we used extrusion processed ginseng to manufacture the wine. Recently, Ha *et al.* (13) reported that the extrusion process is advantageous for the easy release of saponins. Also, Jee *et al.* (5) showed that the saponin extraction rate with extrusion at 155°C was 2 times higher than the saponin extraction rate for an untreated control; while popping decreased the earthy smell of ginseng. However, we found a fermentation delay when we used ginseng that was extrusion cooked at 168°C (Fig. 1a) with a sucrose solution. To identify why the fermentation was delayed, the yeast consumption pattern of the carbon source was compared according to the amount of ginseng extruded at 168°C. When 0-6%(w/v) of the ginseng extrudate was added to the potato dextrose broth, the Brix dropped rapidly to 7 after 3 days of incubation (Fig. 1b). Among the various ginseng concentrations, the alcohol content was highest when 2% ginseng was

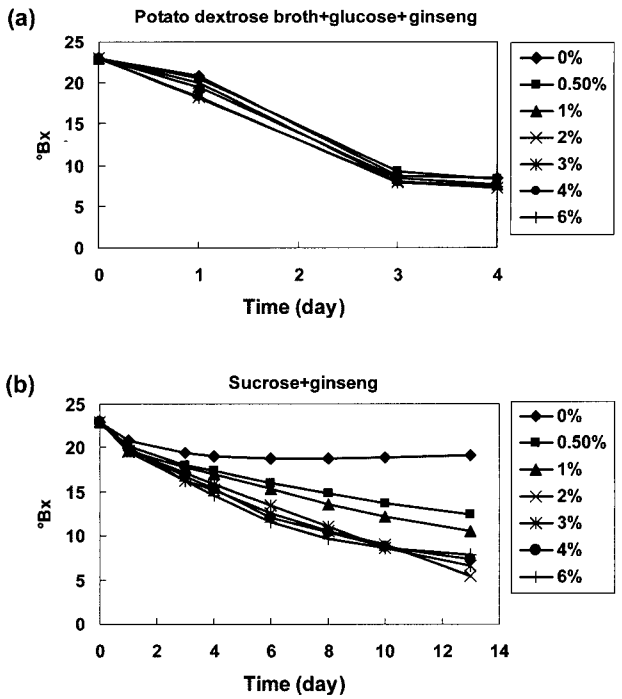

Fig. 1. Ginseng (0-6%) fermentation of 10^8 cells/mL of yeast in sucrose solution (a) and in the yeast growth medium of potato dextrose broth by addition of 23°Bx glucose (b).

used (Table 1). Meanwhile, when the ginseng extrudate was added to a sucrose solution of 23°Bx, the consumption of sucrose was delayed nearly more than 10 days (Fig. 1); therefore, a direct comparison of alcohol content among the extrudate ginseng concentrations was impossible in the sucrose solution experiment. Although there were no differences in the Brix among the 2-6% concentrations of the ginseng extrudate, fermentation was delayed when ginseng extrudates of 0.05 and 1% were used. The final alcohol content reached a maximum of 12% when the Brix was 7. We discovered that the lack of nutrients in the sucrose solution was the reason for the delay in fermentation, since there was no delay when the potato dextrose broth growth medium was used (Fig. 1a).

When various temperatures were applied for the extrusion treatments, temperatures over 160°C triggered a

Table 1. Alcohol content after 3 days fermentation in potato dextrose broth with a sucrose solution of 23°Bx according to the concentration of extruded ginseng cooked at 168°C

Ginseng (%)	Alcohol content (%)
0	11.8
1	11.2
2	12.1
3	9.8
4	9.6
6	8.6

Table 2. The pH of extruded ginseng according to extrusion temperature

	Raw ginseng powder	125°C	160°C	168°C
pH	5.06±0.02	5.05±0.01	5.00±0.03	4.69±0.06

burning smell and delayed fermentation, proving them inappropriate for ginseng wine manufacturing. In our preliminary study, extrudated ginseng that was treated at 125°C was not different from white ginseng in terms of wine fermentation time (data not shown). The sensory characteristics of the extrudated (125°C) wine were superior to those of white ginseng (data not shown). Even when the extrusion temperature of the ginseng was lowered to 75 or 95°C, the wine fermentation times were not different from that of the ginseng extruded at 125°C. However, preferences for the 75 and 95°C extrudated ginseng wines were lower than that of the 125°C extrudated wine (data not shown). The extrusion-treated ginseng in this study was acidic in the range of pH 4.6-5.1 (Table 2). Since it is known that a pH of 6-7 is the optimum pH of yeast (14), the acidic pH of the ginseng extrudate could be one of the factors that delayed yeast fermentation. To minimize the problem of low pH, the ginseng extrudate cooked at 125°C was selected. Also, higher extrusion temperatures can increase MRPs, which may act as chelating agents to the yeast enzyme, decreasing enzyme activity (11, 15).

The effects of the carbon source and nitrogen source on ginseng wine fermentation To compare the effects of the carbon source on ginseng wine fermentation, 2%(w/v) of the selected ginseng extrudate heated at 125°C was added to a sucrose or glucose solution of 23°Bx, and then fermented at 15°C to prevent the growth of other microorganisms. At this time, 0.5% DAP was added to the glucose or sucrose solution and the fermentation time was compared to the glucose and sucrose solutions without DAP (Fig. 2). It took more than 30 days for the Brix to drop from 23 to 7 in the solutions where no DAP added. However, when 0.5% DAP was added, the fermentation time was reduced from 33 to 10 days (glucose solution) and from over 33 to 13 days (sucrose solution). With or without DAP, glucose was consumed more quickly than sucrose by the yeast. The fermentation time was shorter when glucose was used versus sucrose, however, the ginseng wine product acquired a clearer flavor and taste when sucrose, rather than glucose, was used (data not shown). This is supported by the results of Yalçin and Özbas (16), where wine yeast growth was accelerated with glucose rather than the sucrose substrate.

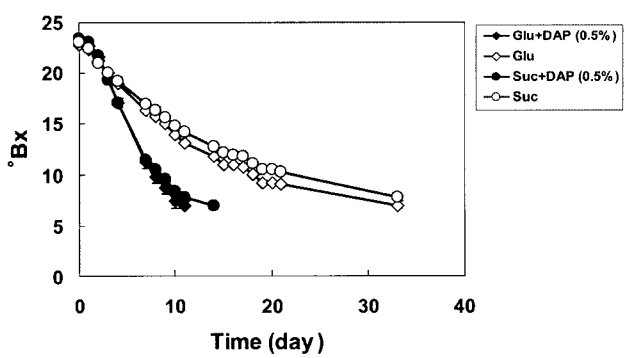

Fig. 2. Ginseng wine fermentation with or without DAP in glucose or sucrose. Ginseng extrudate (extrusion temp: 125°C) and 0.02%(w/v) yeast were mixed in a carbon source solution (glucose or sucrose of 23°Bx) with or without DAP (0.5%).

The addition of DAP as a nitrogen source shortened fermentation time up to 2-fold; however, bitterness was stronger in the extrudated ginseng wine product (data not shown). Therefore, adding DAP to ginseng wine seems inappropriate for the taste, which is different from grape wine. The bitterness may be attributed to the ginseng itself, due to the bitter taste of the saponins (17), as well to a changed flavor caused by the addition of DAP, such as sulfuric and citric flavors (18).

The effects of fermentation temperature, inoculated yeast concentration, and ginseng concentration on the fermentation time and yeast cell number during fermentation To manufacture ginseng wine of good taste and flavor, fermentation without DAP was performed using ginseng that was extrusion-cooked at 125°C along with a sucrose solution. Also, to find a methodology that solves the problem of fermentation delay, we examined various yeast concentrations, ginseng concentrations, and fermentation temperatures. Here, we compared the effects of the fermentation temperature (15, 30°C), yeast inoculum (0.02, 1%, w/v), and ginseng extrudate (1, 2%, w/v) on fermentation time, as monitored by Brix reduction (Fig. 3). In general, a yeast inoculum of 0.02% has been recommended by the wine industry (19), and we have found that a ginseng extrudate over 1% is not appropriate for manufacturing ginseng wine because such concentrations induce strong bitterness and a strong ginseng flavor (data not shown).

When the yeast inoculum and ginseng concentrations were the same, a fermentation temperature of 30°C reduced the ginseng wine fermentation time 4- to 7-fold compared to 15°C. The basic fermentation condition of 0.02% yeast inoculum and 1% ginseng at 15°C did not complete fermentation, even after 30 days (Fig. 3a). The higher ginseng extrudate amount of 2%(w/v) reduced the fermentation time more than the ginseng extrudate of 1%, by more than 5 days, except in the case of the fermentation with the higher yeast inoculum at 30°C (Fig. 3b) The higher yeast inoculum amount of 1%(w/v) shortened the wine fermentation time more than the 0.02% concentration (10^6 cells/mL) by nearly 2-fold, at both 15 and 30°C (Fig. 3). Changing the fermentation temperature from 15 to 30 °C had a greater affect on the fermentation time than changing the yeast inoculum from 0.02 to 1%, or changing the concentration of ginseng from 1 to 2% (Fig. 3). In grape wine, a lack of nutrients at low temperatures such as 15°C can result in a fruity and fresh taste (11); however, it may also cause wine fermentation delay. The addition of 1% yeast resulted in 1.54 log CFU/mL more of yeast than the 0.02% yeast addition (Fig. 4). When a 0.02% amount of yeast was inoculated, yeast growth occurred during the initial 1-2 days. However, the yeast cell number did not

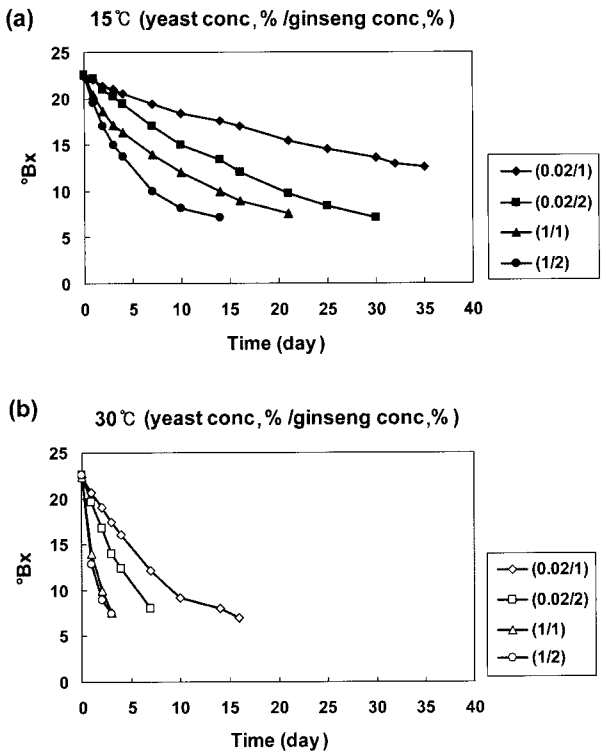

Fig. 3. Ginseng wine fermentation time according to the fermentation temperatures. a) 15°C, closed symbol; b) 30°C, open symbol; dry wine yeast concentrations (0.02%, ◆■; 1%, ▲●) and ginseng concentrations (1%, ◆▲; 2%, ■●).

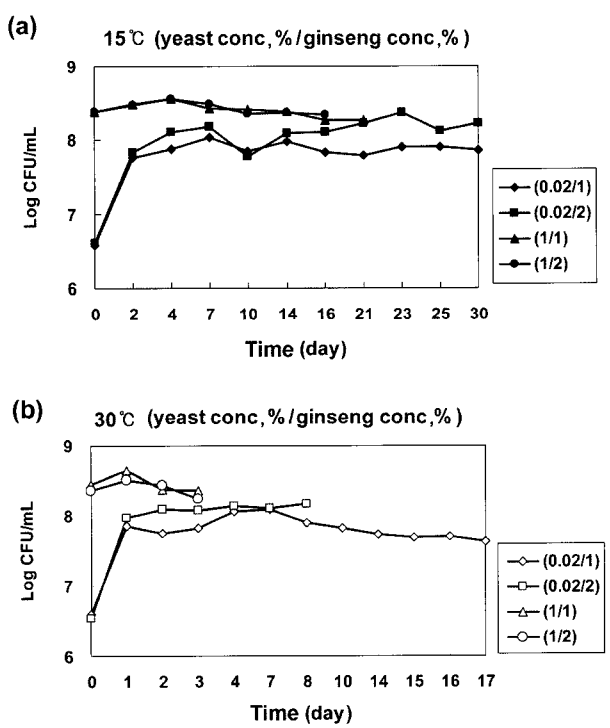

Fig. 4. Viable yeast cell numbers during ginseng wine fermentation according to the fermentation temperatures. a) 15°C, closed symbol; and b) 30°C, open symbol. Dry wine yeast concentrations (0.02%, ◆■; 1%, ▲●) and ginseng concentrations (1%, ◆▲; 2%, ■●) were compared, respectively.

reach that of the 1% yeast at the beginning stage of fermentation (0-2 days). When the 1% yeast was added, there was no increase, but only the maintenance of cell numbers during fermentation. To reach maximum cell numbers, it took 1 or 4 days at 30°C, while it took 4 or 7 days at 15°C. When the 0.02% yeast was inoculated, a higher cell number was shown with the 2% addition of ginseng than with the 1% addition. However, when the 1% yeast was inoculated, there was no difference in the yeast cell number between the 1 and 2% ginseng extrudates, at both 15 and 30°C (Fig. 4). Therefore, we found that the amount of yeast inoculum affected the cell number rather than the fermentation time in this study.

Although the taste of ginseng wine is important, the focus of this research was on reducing fermentation time and identifying the causes of fermentation delay, rather than on sensory value. Further research will be carried out to manufacture ginseng wine with improved taste and flavor. The results of this study, which are related to the fermentation temperature, carbon source, nitrogen source, yeast inoculum, and biochemical characteristics of the materials used, would be useful for the fermented alcohol industry, especially when thermally processed materials and insufficient nitrogen sources are to be included during yeast fermentation.

Acknowledgments

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References

1. Attele AS, Wu JA, Yuan CS. Ginseng pharmacology. Biochem. Pharmacol. 58: 1685-1693 (1999)
2. Wu J, Zhong JJ. Production of ginseng and its bioactive components in plant cell culture: Current technological and applied aspects. J. Biotechnol. 68: 89-99 (1999)
3. Seog HM, Jung CW, Kim YS, Park HS. Phenolic acids and anti-oxidant activities of wild ginseng (*Panax ginseng* C.A. Meyer) leaves. Food Sci. Biotechnol. 14: 371-374 (2005)
4. Oh CH, Kang PS, Kim JW, Kwon J, Oh SH. Water extracts of cultured mountain ginseng stimulate immune cells and inhibit cancer cell proliferation. Food Sci. Biotechnol 15: 369-373 (2006)
5. Jee HK, Cho YJ, Kim JT, Jang YS, Kim CJ. Increase of solubility of *Ginseng radix* by extrusion cooking. Korean J. Food Sci. Technol. 38: 361-368 (2005)
6. Kim JH, Chung DH, Young JW. The effect of Korean ginseng components on the alcohol fermentation by *Zymomonas mobilis*. Korean J. Appl. Microbiol. Biotechnol. 13: 213-221 (1985)
7. Deng C, Yang X, Zhang X. Rapid determination of panaxynol in a traditional Chinese medicine of *Saposhnikovia divaricata* by pressurized hot water extraction followed by liquid-phase microextration and gas chromatography-mass spectrometry. Talanta 68: 6-11 (2005)
8. Joo HK, Kwon WK. Studies on the utilization of waste ginseng meal. Korean J. Appl. Microbiol. Biotechnol. 7: 191-195 (1979)
9. Tauer A, Elss S, Frischmann M, Tellez P, Pischetsrieder M. Influence of thermally processed carbohydrate/amino acid mixtures on the fermentation by *Saccharomyces cerevisiae*. J. Agr. Food Chem. 52: 2042-2046 (2004)
10. Koutinas AA, Wang R-H, Webb C. Development of a process for the production of nutrient supplements for fermentation based on fungal autolysis. Enzyme Microb. Tech. 36: 629-638 (2005)
11. Marks VD, van der Merwe GK, van Vuuren HJJ. Transcriptional profiling of wine yeast in fermenting grape juice: Regulatory effect of diamonium phosphate. FEMS Yeast Res. 3: 269-287 (2003)

12. Novo MT, Beltran G, Torija MJ, Poblet M, Rozès N, Guliiamón JM, Mas A. Changes in wine yeast storage carbohydrate levels during preadaptation, rehydration, and low temperature fermentations. Int. J. Food Microbiol. 86: 153-161 (2003)
13. Ha DC, Lee JW, Rye GH. Change in ginsenosides and maltol in dried raw ginseng during extrusion process. Food. Sci. Biotechnol. 14: 363-367 (2005)
14. Rhee CH, Park HD. Isolation and the characterization of alcohol fermentation yeasts from Presimmon. Korean J. Appl. Microbiol. Biotechnol. 25: 266-270 (1997)
15. Tan BK, Harris ND. Maillard reaction products inhibit apple polyphenoloxidase. Food Chem. 53: 267-273 (1995)
16. Yalçin SK, Özbas ZY. Effects of different substrates on growth and glycerol production kinetics of a wine yeast strain *Saccharomyces cerevisiae* Narince 3. Process Biochem. 39: 1285-1291 (2004)
17. Heng L, van Koningsveld GA, Ruppen H, van Boekel MAJS, Vincken J-P, Roozen JP, Voragen AGJ. Protein-flavour interactions in relation to development of novel protein foods. Trends Food Sci. Tech. 15: 217-224 (2004)
18. Haernández-Orte P, Ibarz MJ, Cacho J, Ferreíra V. Effect of the addition of ammonium and amino acids to musts of Airen variety on aromatic composition and sensory properties of the obtained wine. Food Chem. 89: 163-174 (2005)
19. Tallandier P, Ortual FR, Fuster A, Strehaiano P. Effect of ammonium concentration on alcoholic fermentation kinetics by wine yeasts for high sugar content. Food Microbiol. 24: 95-100 (2007)