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Optimum Alcohol Fermenting Conditions for Kiwi (*Actinidia chinensis*) Wine

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Abstract The objective of this study was to establish the optimum alcohol fermenting conditions for the processing of kiwi wine and vinegar products. Six yeast strains were examined for their alcohol production from kiwi at 30°C for 72 hr with continuous shaking at 100 rpm. Under these conditions, *Saccharomyces kluyveri* DJ97 produced the highest alcohol content of 10.2%. As the fermentation time extended to 96 hr, the alcohol content reached a maximum of 12.75%. The optimum alcohol fermenting conditions for kiwi fruit were accomplished when kiwi was added to an equal amount of water, inoculated with *S. kluyveri* DJ97 and fermented at 30°C for 96 hr with continuous shaking. The content of soluble solids decreased as the alcohol concentration increased, whereas little change was observed in the pH and titratable acidity during the low temperature aging process. Other alcoholic compounds, such as methanol, isopropanol, *n*-propanol, isobutanol, and isoamylalcohol, tended to increase as fermentation progressed.

Key words : *Actinidia chinensis*, kiwi, alcohol, fermentation.

Introduction

Kiwi is a subtropical, deciduous fruit tree bearing typical climacteric fruit, which is matured and consumed after picking. Kiwi (*Actinidia chinensis* Planch. cv. *Hayward*) is currently cultivated in the southern and Jeju island regions of Korea (1, 2). Most kiwi grown in Korea are of the *Hayward* variety, bearing large fruit with excellent storage ability, taste, and flavor (2-4). This fruit is usually consumed raw or kept frozen for jam, juice, or wine processing (5). It is a rich source of vitamin C and contains more vitamin E and minerals than any other fruit (1). In addition to its nutritional value, kiwi is also distinguished for its brilliant colors in the flesh as well as its distinct flavor which is an exquisite combination of sweetness and sourness. Since its flesh softens easily and loses its flavor during storage, kiwi is mostly consumed fresh (6). Therefore, the deterioration of quality during storage needs to be overcome to expand the domestic consumption of kiwi (7). It is also necessary to diversify processed kiwi products to achieve this purpose. The development of fermented kiwi products would be a good approach for such efforts. Since most studies on kiwi have focused on its properties during processing, such as compositional changes in sliced products, methods of improving storage quality (4, 7-9), proteolytic enzymes (6, 10-12) and physiological characteristics in processed kiwi juice in relation to sterilization and storage temperature (2), little information is available on kiwi fermentation. Therefore, in this study we present the optimum kiwi fermenting conditions for the production of kiwi wine.

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Materials and Methods

Materials Kiwi fruits used in this study were of the *Hayward* variety grown in the Gosung region of Gyeongsangnam-do, harvested in November, 2004 and kept at 0°C thereafter.

Microorganisms To select the best yeast strains for kiwi fermentation, 6 strains were donated from the department of fermentation at Keimyung University: (A) *Saccharomyces kluyveri* DJ97 (KCTC 8842P), (B) *Zigosacchromyces cerevisiae* JK99, (C) *Saccharomyces cerevisiae* OMK, (D) *S. cerevisiae* 9, (E) *S. cerevisiae* W, and (F) *S. cerevisiae* GRJ. These strains were cultured in YPD agar medium (1% yeast extract, 2% peptone, 2% glucose, 2% agar, pH 6.0) at 30°C for 24 hr, and kept at 4°C until used.

Proximate composition of kiwi The AOAC method (13) was used to analyze the general composition of kiwi flesh. Water content was measured by the atmospheric pressure heat drying method at 105°C, crude protein content by the Kjeldahl nitrogen quantitative method, crude lipid by the soxhlet extraction method, crude fiber by the fritted glass crucible method, and crude ash by the direct-ashing method. Each content is expressed as a percentage.

Starter culture To initiate the starter culture, 200 g of kiwi homogenate was diluted with 200 mL of water. After sugar was added to 25°Bx, the mixture was sterilized at 121°C for 15 min. Fermentation was initiated by adding one platinum loop of each yeast strain per 10 mL and fermenting in a shaking incubator (HB-201SL; Hanbaek Co., Bucheon, Gyeonggi, Korea) at 30°C for 24 hr.

Optimal conditions for alcohol production In order to select the best yeast strain for kiwi fermentation, 6 yeast

strains were evaluated for their alcohol production by mixing 200 g of kiwi homogenates with 200 mL of water, 10 g of starter culture and 85 g of sugar. The mixture was then cultured in a shaking incubator (HB-201SL; Hanbaek Co., Korea) at 30°C and 100 rpm for 48, 60, 72, 84, and 96 hr. After selecting the best starter culture and fermentation time, 100, 200, 300, 400, and 500 mL of water was added to 200 g of kiwi homogenate to determine the optimum amount of water for alcohol production.

Kiwi wine production Kiwi wine was produced by scaling up the best fermentation process followed by aging. Four kg of kiwi homogenates were mixed with 4 L of water in a 15 L glass instrument and fermented for 96 hr. Thefermented product was then aged at 15°C for 3 months.

Physico-chemical properties of fermented kiwi products The physico-chemical properties such as alcohol concentration, soluble solid, pH, total acidity, and alcohol composition in fermented kiwi products before and after aging were analyzed. Alcohol concentrations were analyzed using the Gay Lussac Table (14) by distilling and adjusting 100 mL of fermented sample to 15°C. Various alcoholic compounds were identified according to the National Tax Service Liquor Analysis Regulation (15) using gas chromatography (Hewlett Packard-6980; Hewlett Packard Co., San Francisco, CA, USA). The analysis conditions were as follows; column: fused silica capillary column (30 m × 0.25 mm), carrier gas: N_2 (60 mL/min), detector: flame-ionization detector (FID), injection temp.: 200°C, detector temp.: 230°C, injection volume: 2 μL. The sugar and soluble solid contents were measured using a refractometer (PR-101; Atago Co., Tokyo, Japan). Total acidity was measured by titrating the sample with 0.1 N NaOH and converting the amount of the base used into the amount of malic acid. Total phenolic compounds in fermented kiwi products aged for 3 months were measured according to the Folin-Denis method (16, 17) with a UV-visible spectrophotometer (UV-1601; Shimadzu, Kyoto, Japan) at 700 nm (18). This value was calculated from a standard curve prepared using tannic acid. The color of kiwi wine was measured using Hunter's color values and UV absorbance at 420 nm. Vitamin C content was analyzed by 2, 4-dinitrophenyl-hydrazine (DNP) according to the food code (18). Organic acid content was analyzed by a HPLC (Waters HPLC 2487; Waters, Milford, MA, USA) with a μ-Bondapak C_{18} column, absorbance at 210 nm, a mobile phase of 10 mM KH_2PO_4 (pH 2.32) and a flow rate of 0.6 mL/min.

Statistical analysis Each experiment was repeated 3 times, and the results were expressed as the mean±SD for the 3 experiments. The alcohol compounds were measured only once.

Results and Discussion

Proximate composition of kiwi Table 1 shows the proximate composition of kiwi fruit. The compositions of water, protein, lipid, ash, and fiber were 82.27, 1.11, 0.32, 0.54, and 2.51%, respectively, which are similar to the results of D'apres's (2).

Optimal conditions for alcohol production
***Screening for yeast strains*:** Fermentation of kiwi was carried out with (A) *S. kluyveri* DJ97, (B) *Z. cerevisiae* JK99, (C) *S. cerevisiae* OMK, (D) *S. cerevisiae* 9, (E) *S. cerevisiae* W, and (F) *S. cerevisiae* GRJ at 30°C and 100 rpm for 72 hr with shaking. The strain that produced the highest alcohol concentration was (A) *S. kluyveri* DJ97 with 10.2%, followed by (B) *Z. cerevisiae* JK99, and (C) *S. cerevisiae* OMK with 9.6% (Fig. 1). The pH and total acidity was similar for all strains (Fig. 2). *S. kluyveri* DJ97 was therefore selected as the optimum strain.
***Fermentation time*:** After 200 mL of water was added to 200 g of kiwi, the sample was cultured for 48, 60, 72, 84, 96, and 108 hr to examine the effects of fermentation time. The alcohol concentration increased gradually as fermentation proceeded, and the highest alcohol concentration of 12.75% was obtained after 96 hr of fermentation with a slight decrease after 108 hr of fermentation (Fig. 3). This

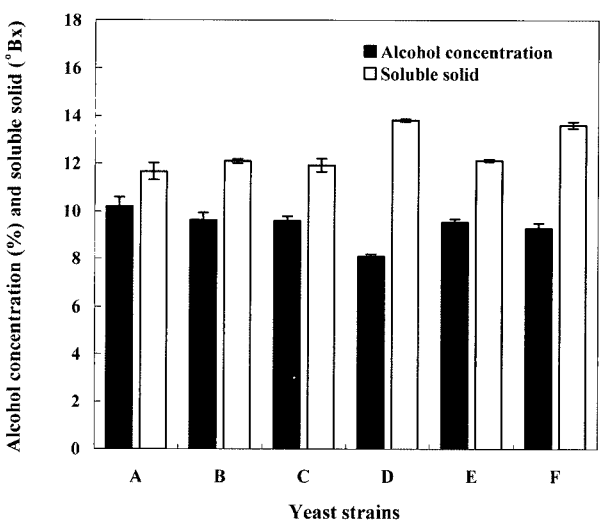

Fig. 1. Changes in alcohol concentration and soluble solids in fermented kiwi with different yeast strains. A: *S. kluyveri* DJ97, B: *Z. cerevisiae* JK99, C: *S. cerevisiae* OMK, D: *S. cerevisiae* 9, E: *S. cerevisiae* W, F: *S. cerevisiae* GRJ. Values are means±SD (n=3).

Table 1. Proximate composition of kiwi

Composition (%, dry weight basis)				
Moisture	Crude protein	Crude fat	Crude ash	Crude fiber
82.27±0.65	1.11±0.23	0.32±0.05	0.54±0.04	2.51±0.07

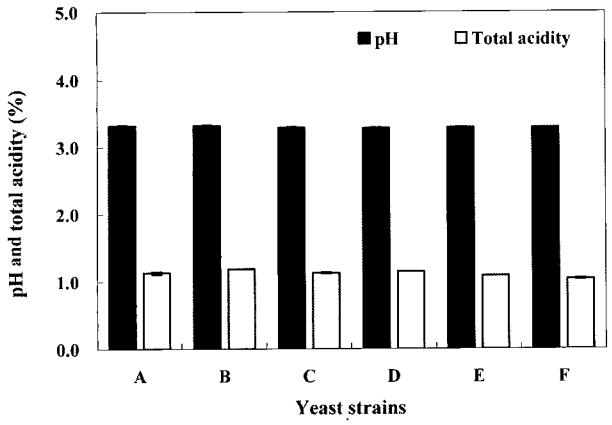

Fig. 2. Changes in pH and total acidity of fermented kiwi with different yeast strains. A, *S. kluyveri* DJ97; B, *Z. cerevisiae* JK99; C, *S. cerevisiae* OMK; D, *S. cerevisiae* 9, E: *S. cerevisiae* W; F, *S. cerevisiae* GRJ. Values are means±SD (n=3).

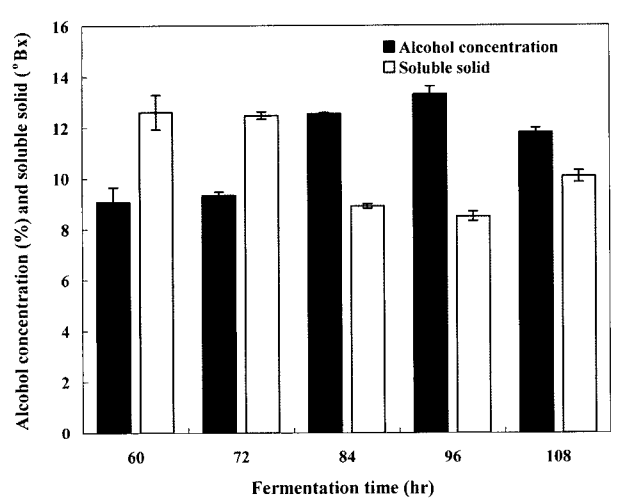

Fig. 3. Changes in alcohol concentration and soluble solids in kiwi fermented by *S. kluyveri* DJ97 for different fermentation times. Values are means±SD (n=3).

result differs from that of Son *et al.* (19) who reported that the alcohol concentration increased until 60 hr of fermentation for Prunus Mume, but did not change significantly thereafter. This difference is probably due to the different materials and different strains used for fermentation. During fermentation, the residual sugar concentration decreased as the alcohol concentration increased, whereas the pH and total acidity did not show any significant changes (Fig. 4).

***Additional water content*:** Figure 5 shows the effects of added water on alcohol fermentation when 50 (100 mL), 100 (200 mL), 150 (300 mL), 200 (400 mL), and 250% (500 mL) volumes of water were added to 200 g of kiwi. The alcohol concentration reached a maximum of 12.37% when an equal volume (100%) of water was added, whereas it decreased as the amount of added water increased. The residual sugar concentration decreased as the alcohol concentration increased during fermentation, showing the lowest value of 9.6°Bx with an equal volume

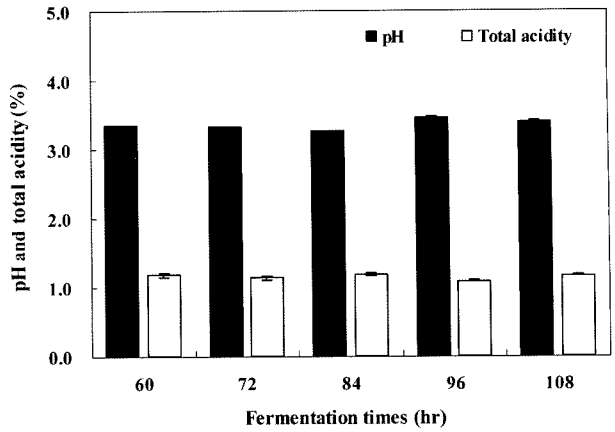

Fig. 4. Changes in pH and titratable acidity in kiwi fermented by *S. kluyveri* DJ97 for different fermentation times. Values are means±SD (n=3).

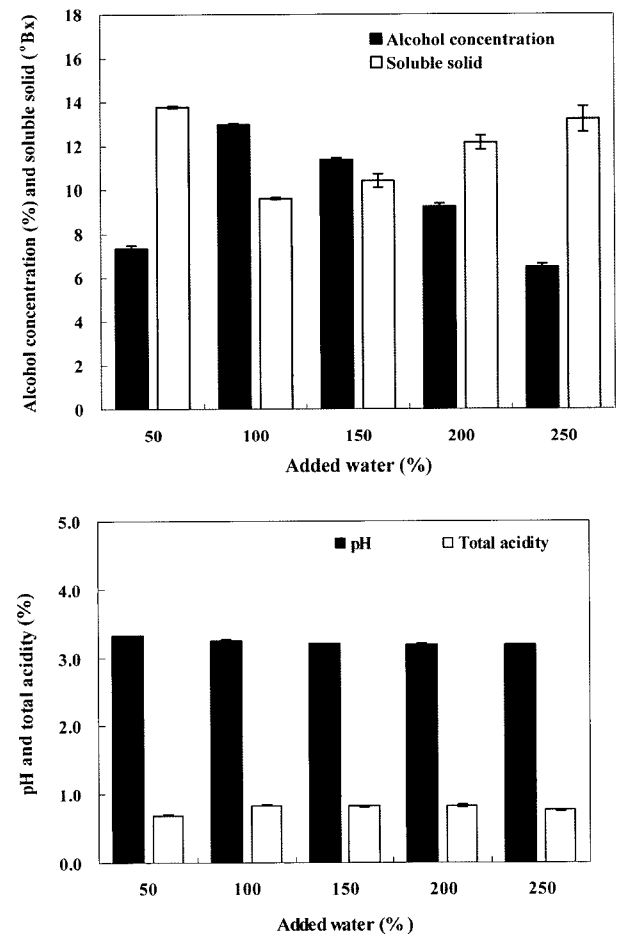

Fig. 5. Changes in alcohol concentration, soluble solids, pH, and total acidity during kiwi fermentation by *S. kluyveri* DJ97 with different amounts of water. Values are means±SD (n=3).

of added water. Different amounts of added water did not have a significant effect on the pH and total acidity. Since the alcohol concentration decreased with additional water above an equal volume, an equal volume of water is optimum for kiwi wine fermentation. This result is similar

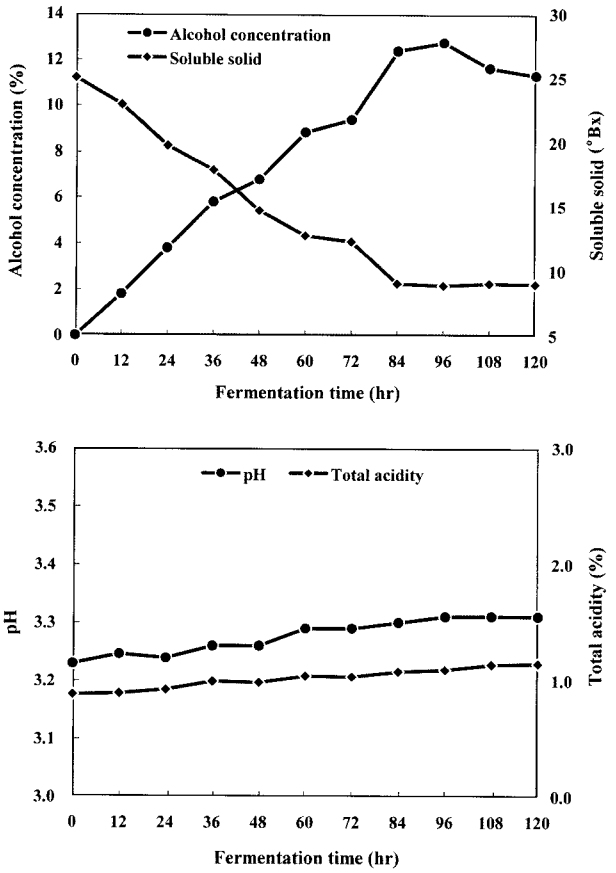

Fig. 6. Changes in alcohol concentration, soluble solids, pH, and total acidity during kiwi fermentation by *S. kluyveri* DJ97.

to that of Jung *et al.* (20) who reported that the alcohol content increased with additional water when a 200, 250, and 300%(v/w) volume of water was added to apricots for fermentation.

Changes in composition during fermentation Figure 6 shows the changes in alcohol concentration and soluble solids during kiwi fermentation. The alcohol concentration increased steadily during fermentation and reached its peak by 96 hr of fermentation at 12.7%, then decreased slightly. The soluble solids were 25.0°Bx at the early stages of fermentation but decreased to 9.0°Bx after 96 hr with no change afterwards. Thus, the optimum fermentation period was determined to be about 4 days. This result is similar to that of Lee *et al.* (21) who investigated the alcohol and acidic properties of fermented muskmelon and reported that the alcohol concentration is highest after 83 hr of fermentation. Furthermore, the pH did not change significantly with fermentation time. The total acidity was 0.88% at the early stages of fermentation, increased steadily with fermentation, and was 1.15% after fermentation, which is not considered a significant change.

In addition to ethanol, some changes were observed in the methanol, isopropanol, *n*-propanol, isobutanol, and isoamylalcohol and acetaldehyde contents during fermentation (Table 2). The acetaldehyde content was 621.49 ppm at the early stages of fermentation but decreased with time. The methanol content increased with fermentation time but did not exceed the methanol content specified by the Korea Component Standards (less than 1,000 ppm) (22). This level was significantly lower than the 485-768 ppm reported by Soufleros *et al.* (23), probably due to differences in the kiwi variety and the fermentation method. The isopropanol, ethylacetate, *n*-propanol, and isobutanol contents were minimal. The isoamylalcohol content decreased with fermentation time, whereas the *n*-amylalcohol content increased.

Physico-chemical properties of kiwi wine Kiwi fermentation was carried out under the optimized fermentation conditions using 4 kg of kiwi, 4 L of water, added sugar to 25°Bx and inoculation with *S. kluyvery* DJ97. After fermentation, the sample was filtered and matured for 3 months at 15°C. The physico-chemical properties of kiwi wine are shown in Table 3. The alcohol concentration and soluble solids were similar to the wine before aging, however the pH was slightly higher while the total acidity was slightly lower. As for color, the L, a, and b values were 97.67, -1.81, and 8.81, respectively. The

Table 2. Changes in alcohol and acetaldehyde contents during kiwi fermentation (ppm)

Components	Fermentation time (hr)									
	12	24	36	48	60	72	84	96	108	120
Acetaldehyde	621.49	514.19	370.57	361.51	276.26	250.22	214.24	238.61	100.11	131.88
Methanol	15.26	18.37	19.26	22.41	26.32	20.98	26.89	39.48	47.39	101.39
Ethanol[1)]	1.88	3.80	5.80	6.77	8.83	9.37	12.37	12.73	12.63	11.30
Isopropanol	ND[2)]	ND	ND	ND	ND	ND	ND	ND	ND	Trace
Ethylacetate	ND	ND	ND	ND	ND	ND	Trace	Trace	Trace	Trace
n-Propanol	ND	Trace	Trace	Trace	Trace	Trace	Trace	9.08	16.46	18.85
Isobutanol	ND	ND	47.74	57.54	trace	ND	ND	ND	ND	ND
Isoamylalcohol	65.35	56.54	37.36	31.79	26.05	14.05	14.57	15.59	13.83	10.15
n-Amylalcohol	16.60	32.95	99.37	112.34	122.06	154.21	174.26	260.40	296.61	460.99

[1)]Unit: %.
[2)]Not detected.

Table 3. Quality characteristics of kiwi wine[1)]

Alcohol concentration (%)		12.35±0.06
Soluble solid (°Bx)		8.95±0.05
pH		3.73±0.01
Total acidity (%)		0.84±0.02
Brown color		0.13±0.03
Hunter's color	L	97.67±0.06
	a	-1.81±0.04
	b	8.81±0.02
Total phenol content (mg%)		64.62±0.12
Total vitamin C (mg%)		76.94±1.56
Organic acid content (ppm)	Citric acid	2,920.11±42.43
	Malic acid	4,370.54±212.21
	Succinic acid	1,540.30±28.28
	Total	8,830.23±226.27

[1)]Values are means±SD (n=3).

phenol content was 64.62 mg%, which is within the 24-200 mg% range reported by Lee and Koh (24) and Koh *et al.* (25) who compared phenol content in Korean grape wines. The vitamin C content was 76.94 mg%, suggesting that vitamin C in the kiwi itself was largely preserved throughout the fermentation process. Regarding organic acids, the malic acid content was highest at 4,385.54 ppm, followed by citric acid and succinic acid at 2,920.11 and 1,540.30 ppm, respectively. The malic acid content is similar to 4,000 ppm reported by Lee *et al.* (26) in grape wine.

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