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Utilization of Gelatin Hydrolysate in White Wine-Making*

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Koshu white wine was made from the musts treated with a gelatin hydrolysate prior to the addition of yeast. Analyses and sensory evaluation showed that the gelatin hydrolysate was a possible material for sulfur dioxide to prevent browning, other oxidative reactions, and formation of cloudiness. The phenol content in that wine, particularly the tannin content, was smaller than that in the wine, made from the musts treated with sulfur dioxide. The peptide content, however, was similar to that in the wine made from the musts treated with sulfur dioxide. There was no appreciable difference in their chemical properties other than the phenol content between the wine made from the musts treated with the gelatin hydrolysate and that made from the must treated with sulfur dioxide.

Sulfur dioxide has been used in must and wine for its antiseptic and antioxidant powers. The powers are known as to decrease its combination with aldehydes, sugars, or other constituents in must and wine since the bound forms of sulfur dioxide are not active as is the free form. The odor of free sulfur dioxide, however, is pungent and it has a negative quality, hence many researchers have searched for a substitute for sulfur dioxide. In case that ascorbic acid is used as one of the antioxidants, it has very little effect on controlling undesirable microorganisms and does not bind acetoaldehyde as sulfur dioxide does. Furthermore, it has been well known that a butter-like and a geranium-like off-odors occured in wine with excessive use of ascorbic acid. Therefore, at present there is no desirable substitute for sulfur dioxide.

Gelatin has been commonly used as a fining agent for wine, especially for red wine to stabilize and clarify a wine with an unstable colloidal cloud caused by excessive tannin in wine. Recently, however, gelatin is hardly used for fining of white wine, since it has poor solubility in water, and the presence of contaminants in it may produce off-flavor or off-odor in wine, and overfining and the secondary formation of cloudiness may be caused by addition of gelatin.

We treated Koshu must with a gelatin hydrolysate prior to the addition of wine yeast in order to remove excessive tannin, and to search for a possible substitute for sulfur dioxide, or to use lower level of dose of sulfur dioxide. The results of this experiment show that a gelatin hydrolysate may be a substitute for sulfur dioxide, therefore, this work gives a new method for wine-making with the addition of the gelatin hydrolysate to musts prior to fermentation.

Materials and Methods

Must and wine Koshu grapes were obtained

* Chemical Studies on Coloring and Flavoring Substances in Japanese Grapes and Wines (XII).

from the Institute Vineyard in 1979. The Koshu must was obtained with a Garolla crusher and a Vaslin-type press. About 65 ℓ of the must was obtained from 100 kg of the grapes. A gelatin hydrolysate or sulfur dioxide (as potassium metabisulfite) was added to the must in various concentrations. Then, wine yeast (*Saccharomyces cerevisiae* OC-2) was immediately added to the musts treated with the above two materials. Racking was done twice at a month and six months after the addition of the yeast, then the wines were bottled and stored at 15℃ for one year. General analyses of the wines were carried out according to the methods in "*Jozo Bunsekiho*."[1)]

The gelatin hydrolysate (PE 20) used was kindly supplied by Nippi Inc. (Tokyo). The hydrolysate was obtained by enzymatic hydrolysis with a protease, Miyapron (Teikoku Koso, Tokyo).

Amino acids, peptides, and nonvolatile acids The wines were centrifuged at 40,000 rpm for 30 min at 15°C. The supernatant was concentrated under reduced pressure at 40°C and lyophilized. The contents of amino acids in both the lyophilized materials and their acid hydrolysates (hydrolyzed in 6 N HCl for 24 hr at 110°C) were determined with a JEOL Model JLC-6AH amino acid analyzer. The amino acids constituting the total peptides were estimated from the differences between the values thus determined.

Nonvolatile acids were analyzed by high performance liquid chromatography. The samples were applied to a column (0.8 × 50 cm) of Shimadzu gel SCR 101 without pre-treatment, equilibrated with a pH 1.9 aqueous solution (adjusted with perchloric acid). The column was eluted with the same solution at a flow rate of 1.0 mℓ/min.

Browning, turbidity, and concentration of phenolic compounds Degree of browning was measured by optical density at 400 nm. Degree of turbidity was determined with a turbidmeter (Corona UT-11) and was expressed as unit. Analyses of phenolic compounds were carried out as follows. The wine samples were adjusted to pH 2.0 with dilute hydrochloric acid. To 5 mℓ of the wine in a test tube with a glass stopper was added an equal volume of ether saturated with distilled water and the mixture was shaken, then allowed to stand. The upper phase was removed and the lower phase was extracted twice more in the same way. The lower phase remaining after the extraction with ether was extracted three times with an equal volume of butanol-2 saturated with distilled water as described above. The combined upper phases were extracted with an equal volume of benzene. The aqueous phase was dried by rotary evaporation *in vacuo* at 30°C. To the residue was added 1 mℓ of 0.1 N HCl, and the mixture was heated in boiling water for 20 min. The resulting solution was extracted three times with 1 mℓ of ether. The combined ether extracts and the aqueous layer were dried. Each residue was dissolved in 5 mℓ of water and their phenolic contents were determined by the modified Folin-Ciocalteu method[2)] The ether-extractable phenolics were non-tannin phenolics and most of the phenolics in the aqueous phase were tannin.[3)]

Results and Discussion

Koshu white wines were made from the musts treated with sulfur dioxide (150 ppm), the gelatin hydrolysate (100 ppm to 750 ppm), and a combined dose of sulfur dioxide (75 ppm) and the gelatin hydrolysate (75 ppm). The number average molecular weight of the hydrolysate was about 2,000 according to the personal communication with Nippi. The hydrolysate was able to be easily dissolved in water without heating and was colorless. Various Koshu wines made from the musts treated with the above materials were not much different from one another in specific gravity, and contents of extract and ethanol (Table 1).

Table 1. Specific gravity and contents of extract and ethanol.

Wines		Specific gravity	Extract	Ethanol
Sulfur dioxide	150 ppm	0.9900	2.00%	13.2
Gelatin hydrolysate	100 ppm	0.9900	2.06	13.4
	150 ppm	0.9900	2.13	13.6
	500 ppm	0.9900	2.06	13.4
	750 ppm	0.9900	2.24	14.0
Sulfur dioxide 75 ppm + gelatin hydrolysate 75 ppm		0.9900	2.08	13.5

Table 2. Contents of free amino acids and nonvolatile acids.

Wines		Free amino acids mg/ℓ	Nonvolatile acids			
			Citric acid	Tartaric acid g/ℓ	Malic acid	Acetic acid
Sulfur dioxide	150 ppm	867	0.68	2.39	1.83	0.29
Gelatin hydrolysate	100 ppm	791	0.66	2.42	1.47	0.51
	150 ppm	804	0.40	2.33	1.25	0.50
	500 ppm	880	0.70	2.47	1.32	0.61
	750 ppm	1054	0.51	2.60	1.48	0.49
Sulfur dioxide 75 ppm + gelatin hydrolysate 75 ppm		884	0.61	2.37	1.32	0.97

The proteins present in the Koshu wine were 0.1 to 0.4 mg/ℓ.[4] The values were considerably small compared with those of the total free amino acids and the peptides (989 mg and 137 mg, respectively) in the wine.[5] Therefore, the wine samples were not deproteinized when we determined the contents of free amino acids and peptides.

The free amino acid contents in the wines with the hydrolysate were not much different from those of the wine with only sulfur dioxide with the exception of those in the wine with 750 ppm of the hydrolysate. The contents and compositions of nonvolatile acids show in Table 2. In each nonvolatile acid, the contents in the six wine samples were rather similar to one another.

The peptide contents in the wines with 100 ppm and 150 ppm of the hydrolysate, and both 75 ppm of the hydrolysate and 75 ppm of sulfur dioxide were 113.4 mg, 101.5 mg, and 112.1 mg, respectively (Table 3). These values were similar to the value in the wine with only sulfur dioxide. The compositions of amino acids constituting the peptides in the wines other than the two wines with 500 ppm and 750 ppm of the hydrolysate added at the concentration of 100 ppm or 150 ppm were removed as precipitate or assimilated by yeast as nitrogen source during fermentation and aging.

The addition of the hydrolysate at the concentration of 100 ppm or 150 ppm decreased the degree of browning, and it did not affect turbidity formation. It appears that turbidity and browning are primarily affected by phenol contents and their oxidation, namely, the phenols are the substrate for browning and turbidity formation. The phenol contents,

Table 3. Compositions of amino acids constituting peptides.

	Wines					
	Sulfur dioxide 150 ppm	Gelatin hydrolysate 100 ppm	Gelatin hydrolysate 150 ppm	Gelatin hydrolysate 500 ppm	Gelatin hydrolysate 750 ppm	Sulfur dioxide 75 ppm + Gelatin hydrolysate 75 ppm
			(mg/ℓ)			
Asp	20.2	24.2	21.0	49.9	51.1	28.2
Thr + Ser	4.3	4.1	1.3	9.5	4.9	3.9
Glu	6.9	9.9	7.9	17.1	19.1	11.2
Pro	trace	trace	trace	trace	trace	trace
Gly	9.0	7.3	9.7	39.7	25.7	6.5
Ala	7.3	3.0	7.8	16.3	30.5	6.4
Val	9.3	13.2	9.0	18.4	19.7	8.5
Met	1.9	trace	1.6	5.2	4.6	trace
Ile	6.7	6.6	5.9	12.9	14.7	6.3
Leu	7.5	5.4	5.6	12.6	16.0	7.0
Tyr	3.4	7.4	5.1	9.1	15.0	5.1
Phe	3.5	7.4	5.1	9.1	15.0	5.1
His [1]	10.5	13.3	14.5	15.0	18.7	9.4
Lys	10.8	10.4	6.0	18.0	22.5	10.1
Arg	5.1	6.4	6.1	13.6	14.3	6.8
Total	106.4	113.4	101.5	240.7	256.8	112.1

1) These values include histidine and at least two unknown ninhydrin-positive compounds.

Tadle 4. Browning, turbidity, and contents of ether-extractable nontannin phenolics and butanol-2-extractable tannins.

Wines		Absorbance at 400 nm	Turbidity (units)	Ether-extractable non-tannin phenolics (mg/ℓ) [1]	Butanol-2-extractable tannins (mg/ℓ) [2]
Sulfur dioxide	150 ppm	0.145	0.22	38.0	102.0
Gelatin hydrolysate	100 ppm	0.105	0.17	23.5	61.5
	150 ppm	0.115	0.24	33.0	72.0
	500 ppm	0.130	0.36	31.5	73.5
	750 ppm	0.145	0.46	39.0	81.0
Sulfur dioxide 75 ppm + gelatin hydrolysate 75 ppm		0.100	0.05	37.5	75.0

1) as gallic acid equivalent.
2) as catechin equivalent.

especially the tannin contents in the wines with the hydrolysate were smaller than those in the wine with only sulfur dioxide (Table 4). The hydrolysate was found to bind with ease to many phenols such as catechin, dimeric phenols, and tannins, but its binding ability to the tannin was poorer than that of the unhydrolyzed gelatin.[6] Therefore, there seemed to be little danger of overfining even if a large amount of the hydrolysate was used. In fact, the binding of the hydrolysate to the tannins in the must or the wines during fermentation and aging seemed to occur resulting in removal of a limited amount of tannins.

The wines made from the musts with the hydrolysate (100 ppm or 150 ppm) were slightly brownish but not cloudy. The sensory impression of the aftertaste of the wines was fresh, but they had no olfactory characteristic of bouquet and aroma. No off-flavors or off-odors were perceived. These sensory impressions are common in Koshu wine. However, the wines with the hydrolysate of 500 ppm and 750 ppm had a little aldehyde-odor and a slight bitterness. Of the wines made, the best wine in taste and odor was the one obtained with use of the must treated with both sulfur dioxide and the hydrolysate.

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