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Changes in General Constituents and Phenols during Fermentation in Koshu Wine-Making with Gelatin and Gelatin Hydrolysates *

KOKI YOKOTSUKA, TOSHIHIDE MATSUDO, and TADAE KUSHIDA

The Institute of Enology and Viticulture, Yamanashi University, Kofu 400.

Gelatin and gelatin hydrolysates of different molecular weights were added to the must from Koshu grapes prior to the additon of wine yeast. The musts or wines during fermentation were sampled several times over a month, on which various physical and chemical analyses were performed. The addition of the gelatin and gelatin hydrolysates affected the contents of various wine constituents, especially phenols, and the rate and extent of their changes. The phenol-precipitation capacity of the gelatin hydrolysates of a molecular weight more than 5,000 was similar to that of the gelatin of a molecular weight of 100,000.

Gelatin has been used as a fining agent for long. It coagulates and precipitates as a gelatin-tannin complex when added to wine. This coagulation or precipitation is due to a chemical combination of gelatin and tannin. The weight of tannin precipitated varies according to the sources and the isolation methods of gelatin.[1-7] Gelatins of various kinds have been used up to the present and are commercially available now, but no study of the use of hydrolysis products of gelatins in wine-making has ever been reported except for our previous study.[8] We showed, in the previous study, that the addition of a gelatin hydrolysate of molecular weight of about 2,000 to Koshu must reduced the phenol content of the resulting wine appreciably while no change in the contents of other wine constituents was observed.

In this paper, we used cattle-bone gelatin and its hydrolysates of different molecular weights. They were added to the Koshu must prior to the addition of wine yeast and changes in various wine constituents during fermentation were investigated.

Materials and Methods

Must and wine Koshu grapes were obtained from the Institute Vineyard in 1981. The must (about 200 *l*) was obtained from the grapes with a Garolla crusher and a Vaslin-type press. Sulfur dioxide (30g as potassium metabisulfite) was added to the must and the must was allowed to settle overnight. The sugar content of the must was measured with a Atago refractometer and the reading was 14.5°. Sucrose was added to the must to give the reading of 24°.

To this, the culture solution (4 *l*) of wine yeast (*Saccharomyces cerevisiae W-3*) was added. Immediately after the addition of the yeast, gelatin or gelatin hydrolysate was added to the mixture to give the final concentration of 500mg/*l*. The gelatin and gelatin hydrolysates used were kindly

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supplied by Nippi Inc. (Tokyo). The gelatin was isolated from cattle bone by an alkali method. The gelatin hydrolysates, PA 10 and PA 100, were obtained by acid hydrolysis of the above gelatin with dilute HCl, and the hydrolysates, PE 20 and PE 50, were prepared by enzymatic hydrolysis with a protease, Miyapron (Teikoku Kōso). According to Nippi, the number average molecular weights of the hydrolysates were about 1,000 for PA 10, 2,000 for PE 20, 5,000 for PE 50, and 10,000 for PA 100, and that of the gelatin was about 100,000. Each of the hydrolysates or the gelatin was dissolved in water to give a concentration of 10% just before the addition to the must.

General analyses and determination of contents of total phenol and gelatin or gelatin hydrolysates General analyses of the musts and wines were carried out according to the methods in "Kokuzeichō Shoteibunsekihōchūkai"[9] and "Methods for Analysis of Musts and Wines".[10] All the samples were filtered through a membrane filter (Gelman, alpha-450) before analysis.

Total phenol content was determined by the method of Singleton and Rossi.[11]

The contents of the gelatin or gelatin hydrolysates left in soluble form in the must or wine were determined as follows. An approriate volume of the must or wine made with the gelatin or either of the gelatin hydrolysates was poured into a test tube (14mm × 15cm) and evaporated to dryness. Two m*l* of 6 N HCl was added to the tube. The tube was evacuated, sealed, and kept at 110°C for 24h. After hydrolysis, analyses of amino acid composition were performed with an amino acid analyzer (Kyōwa Seimitsu, Tokyo). The contents of all the amino acids found were summed up. At the same time, the wine made without them was sampled and the amino acid analysis was done as described above. The content of the gelatin or gelatin hydrolysates in the must or wine was estimated from the difference between the values for the two samples.

Results and Discussion

Amounts of gelatin or gelatin hydrolysates to be added In fining a white wine, tannin is added first, followed by gelatin in order to prevent persistent cloudiness and overfining. Less than 100mg each of tannin and gelatin are commonly used per 1 *l* of wine. In this experiment, however, the gelatin or either of the gelatin hydrolysates of different molecular weights was added to the Koshu must in the high concentration (500mg/*l*) prior to the addition of the yeast while no tannin was used, since we expected that the addition of larger amounts of the gelatin or gelatin hydrolysates would present further distinctions of the compositions among the wines made.

Specific gravity and extract A considerable difference in specific gravity was found among the wines sampled from day 5 to day 13 after the addition of the yeast. During this period, the specific gravity of the wines decreased with an increase in the molecular weights of the gelatin hydrolysates (or gelatin) added (Fig. 1).

The extract contents of all the wines, on day 33, were about 2%. From day 5 to day 33, the extract content of the wine made without the gelatin hydrolysates (or gelatin) was larger than that of the wine with them. As observed in the specific gravity, the extract contents of the wines decreased as the molecular weights of the gelatin hydrolysates (or gelatin) increased (Fig. 2).

Reducing sugar and ethanol The addition of the gelatin or gelatin hydrolysates gave the significant effect on the fermentation rates of the musts. The fermentation rate is indicated by the rates of the production of ethanol and the reduction of sugar. In Figs. 3 and 4, the musts treated with the gelatin or gelatin hydrolysates fermented more rapidly than the must without them. Among the wines sampled on day 5 and day 13, the content of the reducing sugar decreased and that of ethanol increased as the molecular weights of the gelatin hydrolysates (or gelatin) increased.

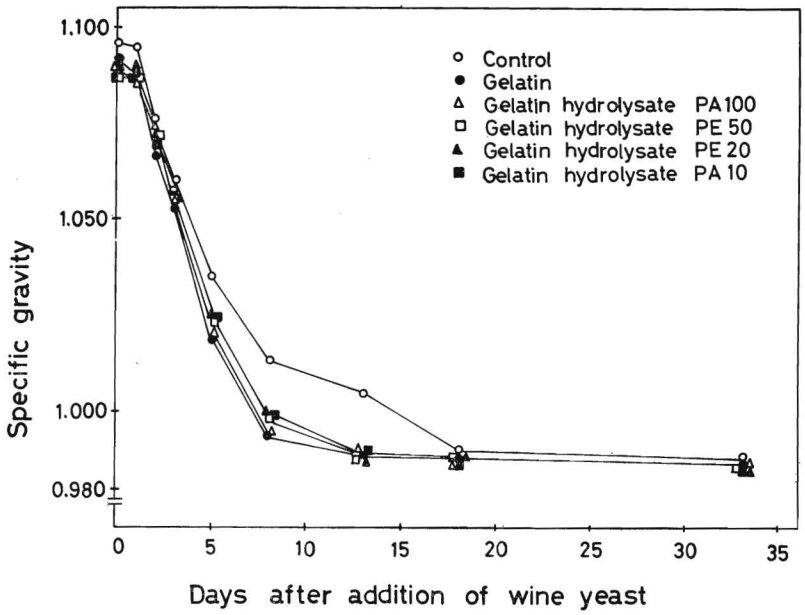

Fig. 1. Changes in specific gravity during fermentation.

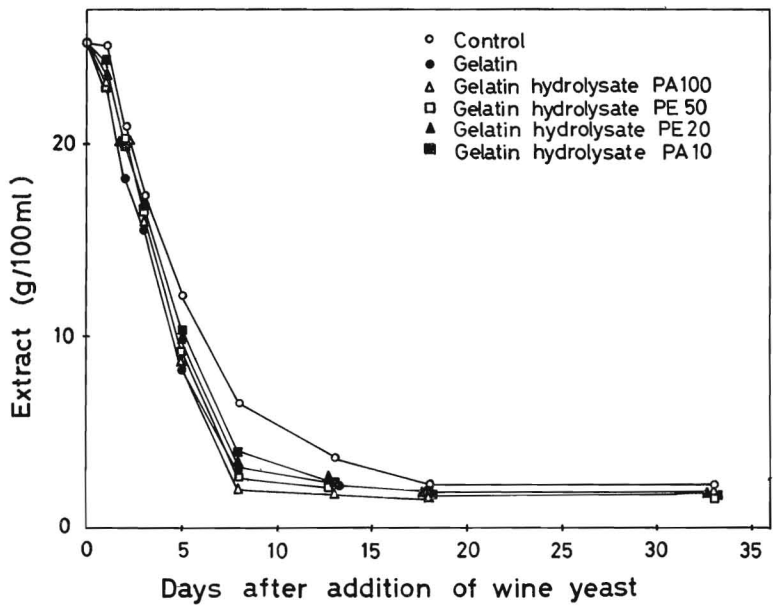

Fig. 2. Changes in extract during fermentation.

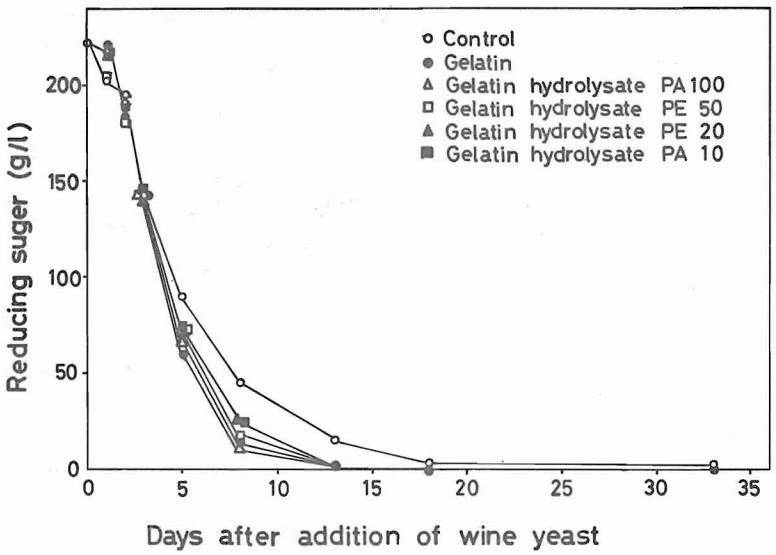

Fig. 3. Changes in reducing sugar during fermentation.

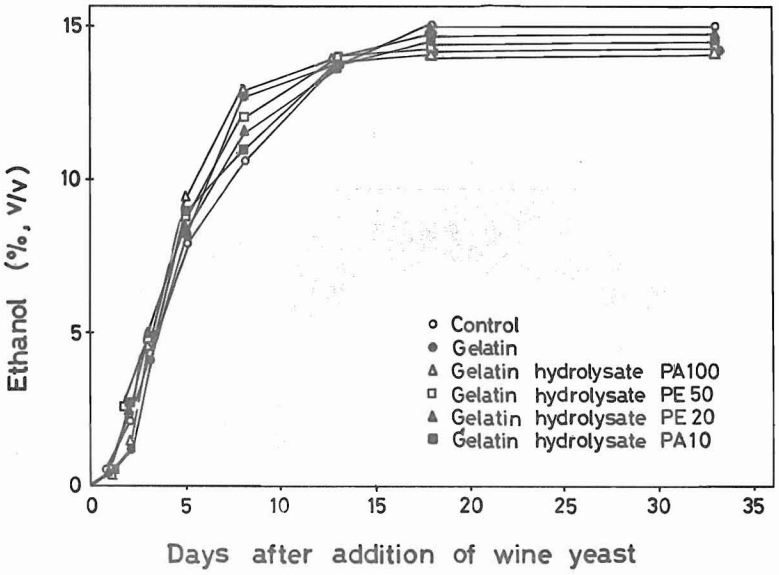

Fig. 4. Changes in ethanol during fermentation.

pH, total acid, and volatile acids The maximum content of total acid and the lowest pH were obtained in the analyses of the wines sampled on either day 2 or day 3 (Figs. 5 and 6). The volatile acid contents of all the wines had increased for 18 days after the addition of the yeast (Fig. 7). No significant differences in pH, total acid, and volatile acids were found among the musts and wines treated with either of various gelatin hydrolysates or gelatin.

Total phenol Figure 8 shows changes in the total phenols of the various wines during fermentation. The total phenol con-

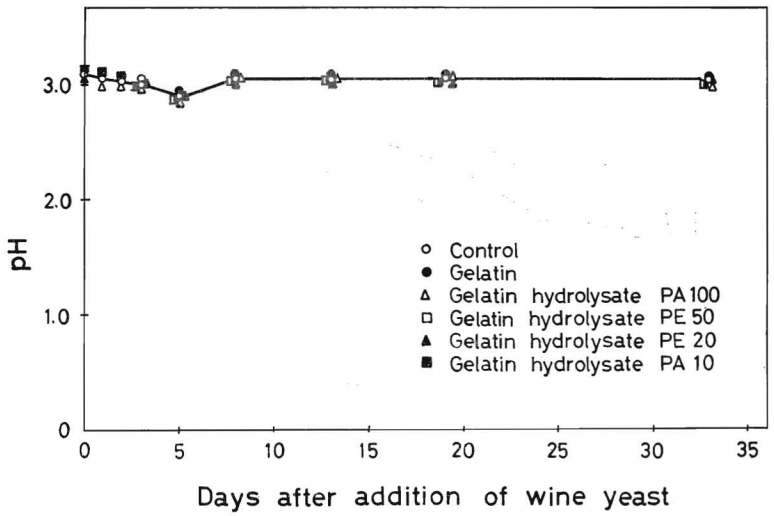

Fig. 5. Changes in pH during fermentation.

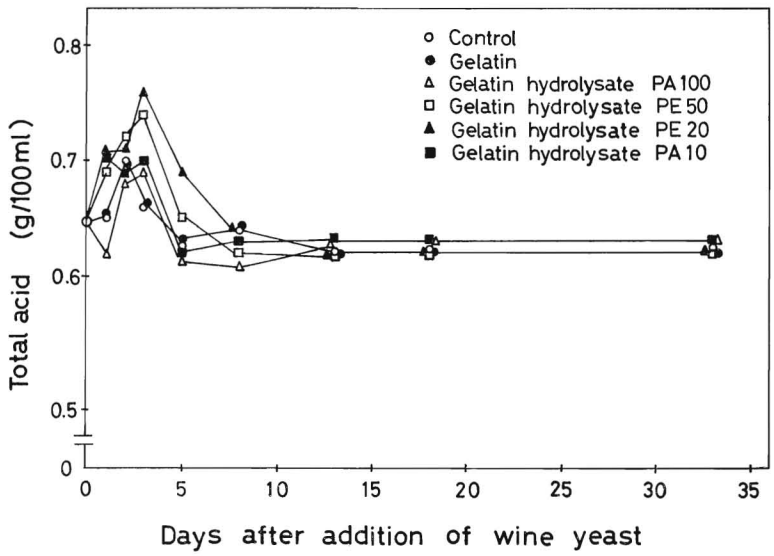

Fig. 6. Changes in total acid during fermentation.

tents of all the wines decreased abruptly for 5 days after the addition of the yeast, and then decreased gradually from day 8 to day 33. Most of the phenols lost seem to be tannins because the monomeric and dimeric phenol fractions from grape seeds were precipitated neither with the gelatin nor gelatin hydrolysates, but the tannin fraction was precipitated with either of them.[12] The total phenol contents of the wines made with the gelatin or the gelatin hydrolysate were smaller than that of the wine made without them when those of the wines sampled on the same day were compared. The molecular weights of the gelatin hydrolysates (or gelatin) added showed significant

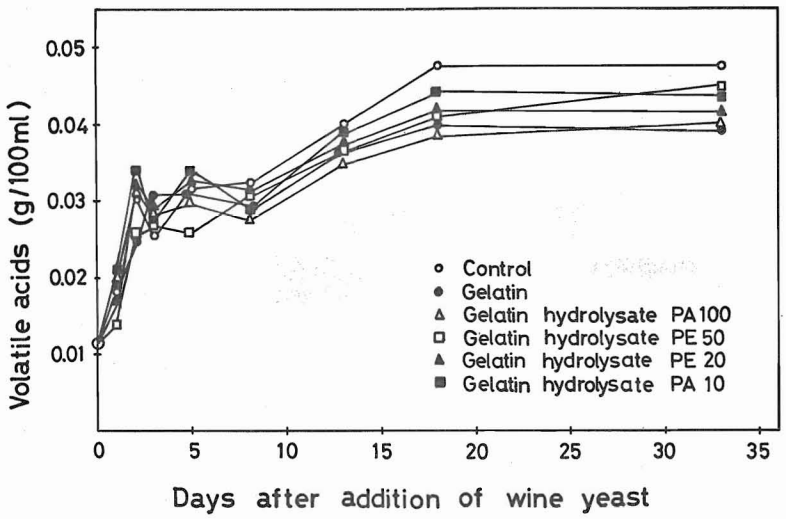

Fig. 7. Changes in volatile acids during fermentation.

influence on the total phenol contents. Among the wines sampled on day 33, the loss of total phenol tended to increase as the molecular weights of them increased.

Gelatin and gelatin hydrolysates

From 65% to 80% of the gelatin or gelatin hydrolysates added were precipitated for 2 days after the addition of the yeast. It was observed on day 33 that 85% to 95% of them had been precipitated. The amounts of the gelatin or gelatin hydrolysates left in each wine hardly changed from day 8 to day 33 after the addition of the yeast (Fig. 9). The amount of the total phenol in each wine sampled on day 18 was similar to that on day 33 as shown in Fig. 8. From these

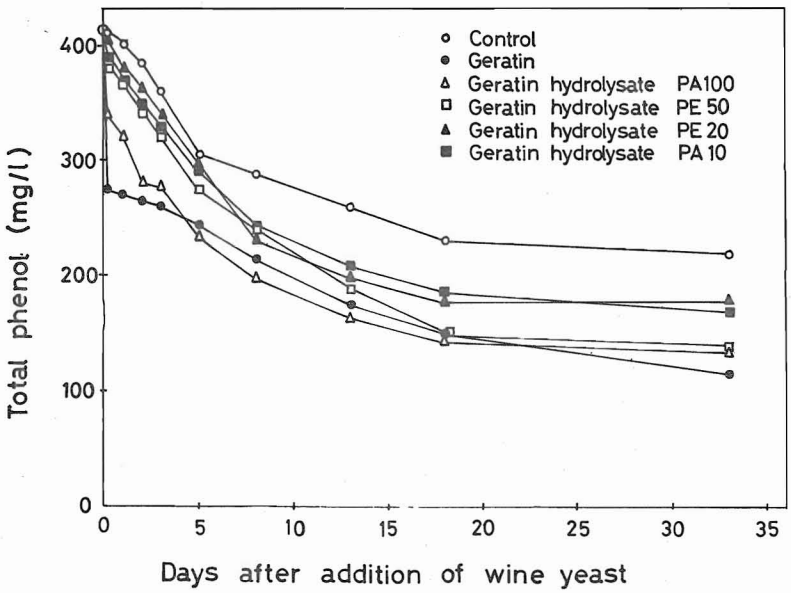

Fig. 8. Changes in total phenol during fermentation.

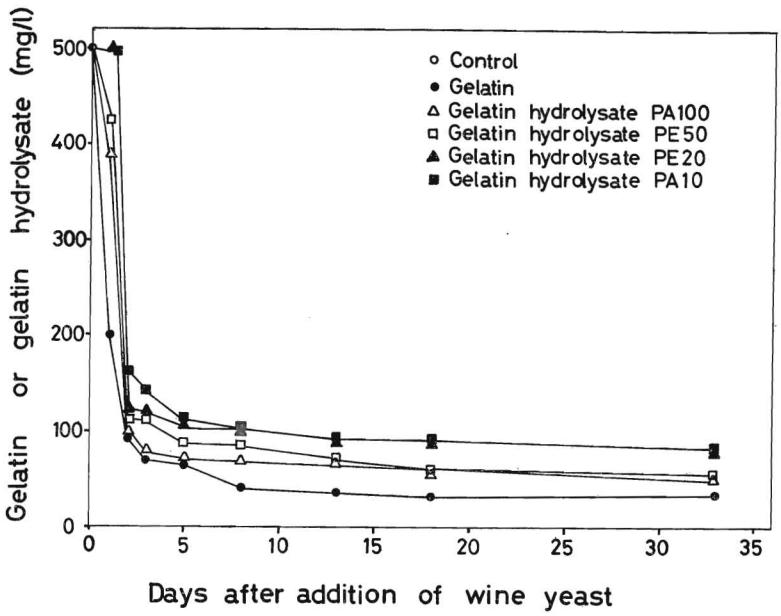

Fig. 9. Changes in gelatin and gelatin hydrolysates added during fermentation.

Table 1. Loss of phenols by gelatin or gelatin hydrolysate.

	Weight of gelatin or glatin hydrolysate precipitated (A) *	Weight of phenols lost from wine (B) *	Weight of phenols lost by gelatin or gelatin hydrolysate [C = B − Control (187)]	Ratio (C/A)
Control	0 **	187 ***	0	—
Gelatin	468 mg/l·wine	273	86	0.18
Gelatin hydrolysate				
PA 100	439	277	90	0.21
PE 50	439	270	83	0.19
PE 20	412	240	53	0.13
PA 10	408	230	43	0.11

* These values were obtained by analyses of the wines sampled at 18 days after the addition of the wine yeast.

** Each gelatin hydrolysate or gelatin was dissolved in the Koshu must to give the final concentration of 500 mg/l.

*** The total phenol content of the original must was 415 mg/l (gallic acid equivalent).

results, the ratio of the weight of the total phenol lost to the weight of the gelatin or gelatin hydrolysate precipitated was calculated from the data on the wines sampled on day 18 in Figs. 8 and 9. The phenol-precipitation capacity of the gelatin hydrolysates PA 100 and PE 50 was very similar to that of the gelatin as indicated by the ratios in Table 1. However, the capacity of the gelatin hydrolysates PE 20 and PA 10 was smaller than that of the gelatin (about 1/2). Thus the loss of phenols was not proportional to the molecular weights of the gelatin hydrolysates (or gelatin) added.

The major conceivable advantages of the use of the gelatin hydrolysates which were confirmed in this experiment are removal of excessive tannins from must, rapid and homogeneous solubilization of the hydrolysates, and no necessity of the use of tannins for fining white wine.

Acknowledgements

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