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## Note

# Koshu Grape Pectins : Isolation, Chemical Composition, and Precipitation *

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Crude pectins were extracted from Koshu grapes with hot water. The pectins were precipitated by ethanol and purified by DEAE-Sephadex A-50 chromatography. The purified pectin contains galacturonic acid (92%), galactose, arabinose, xylose, and rhamnose as polysaccharide components. The degree of esterification was 70%. Various factors influencing the precipitation of the pectin in musts or wines were investigated. The pectin was effectively precipitated at low temperatures, at pH below 3.0 and at more than 7% ethanol concentration. The pectin was also precipitated at the low concentrations of metal ions such as $Cu^{++}$ $Ca^{++}$ $Fe^{++}$ and $K^{+}$ and Koshu must proteins.

Pectins are one of the colloidal constituents of musts and wines. The presence of a large amount of pectins sometimes makes clarification of musts and filtration of wines difficult. Therefore, pectic enzymes have been used for the increase of the yield of free-run juices from musts, or for clarification or filtration of juices or wines. However, the use of the enzymes increases the galacturonic acid and methyl alcohol contents in wines.

Most of must pectins are hydrolyzed by naturally occurring pectic enzymes and precipitated by the alcohol produced during fermentation, hence the concentration of pectins in wines is smaller than that in musts. Pectins and their hydrolysis products act as protective colloids to prevent the precipitation of suspended materials. They may also be bound to various wine components to form haze or sediment. However, the details are still not completely elucidated.

This report describes the isolation of pectins from Koshu grapes, their chemical composition, and factors influencing the precipitation of them.

Koshu grapes were crushed and pressed with a Garolla crusher and a Vaslin-type press. To the pomace (1kg) left in the press, water (2 *l*) was added and boiled for 30min. The pH of the extract was 4.0 to 4.5. The extract was filtered through a Toyo filter No. 101. The filtrate was cooled, concentrated to one-third of its original volume. After the concentrate had been adjusted to pH 1.0 with HCl, 3 volumes of ethanol were added to the concentrate. The precipitate formed was collected by filtra-

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

Table 1. General composition of the crude and purified pectins from Koshu grapes.

| Component | Crude pectin % | Purified pectin % |
|---|---|---|
| Polysaccharide | 79.4 | 97.0 |
| Phenol | 14.5 | 0.8 |
| Ash | 2.5 | 1.8 |
| Others | 3.6 | 0.4 |

Table 2. Composition of polysaccharide components of the purified pectin.

| Components | % |
|---|---|
| Galacturonic acid | 95.4 |
| Arabinose | trace |
| Galactose | 1.8 |
| Rhamnose | 2.8 |
| Xylose | trace |

tion through a Toyo filter No. 2, washed with ethanol and acetone, and dried. The yield of the crude pectin was about 4g. The pectin contents of the must from the Koshu grapes and the wine were about 0.3 g/*l* and 0.06g/*l*, respectively when determined by the carbazole-$H_2SO_4$ method.[1)]

The crude pectin was dissolved in a small amount of water. Insoluble substances were removed by centrifugation. The supernatant was applied to a column of DEAE-Sephadex A-50 (2.3×25cm) equilibrated with 1/100 M phosphate buffer (pH 6.0). The column was washed with the pH 6.0 buffer (350m*l*). Pectins were eluted with 0.1 N HCl. The yield was 1.1g. This pectin fraction was used as the purified pectin fraction in the subsequent experiments.

Tables 1 and 2 show the compositions of the pectins. Polysaccharides and total phenols were determined by the methods of Hodge and Hofreiter,[2)] and Singleton and Rossi,[3)] respectively. Ash content was determined by the method in "Office International de Vigne et du Vin."[4)] The composition of the cations in the ash was analyzed with a Hitachi atomic absorption spectrometer (Model 170-30). The concentrations of cations were 16.2mg/g for $Na^{+}$ 0.33 mg for $Fe^{++}$ 0.16mg for $Mg^{++}$ 0.10mg for $Cu^{++}$ 0.094mg for $K^{+}$ and 0.082mg for $Ca^{++}$

The pectin was hydrolyzed in 0.2 N $H_2SO_4$ at 100°C for 6h. The hydrolysate was neutralized with barium carbonate. The precipitate formed was removed by centrifugation. The supernatant was passed through a column of Amberlite IR-120(1.0 ×10cm). To the eluate, an equal volume of acetone was added, and the mixture was filtered through a Toyo No. 5c filter. The filtrate was evaported to a syrup. This was used as a sample for thin-layer chromatography. Ascending thin-layer chromatography was carried out at room temperature with a solvent system of ethyl acetate : pyridine : acetic acid : water (5 : 5 : 1 : 3, v/v). Avicel cellulose AF (Asahi Kasei, 10g) was vigorously shaken with 35m*l* of water. The slurry produced was poured onto a glass plate (20×20cm). After air drying, the plate was heated at 60° to 80°C for 20min. After the solvent front had reached within about 7cm of the upper edge of the plate, the plate was removed from a chamber and dried under a slow air current. The dried plate was sprayed with the diphenylamine-aniline-phosphoric acid reagent,[5] and heated at 80°C for 4min. The plate was scanned zigzag at 400nm for arabinose, xylose, and rhamnose, 440nm for galacturonic acid, and 640nm for galactose with a Shimadzu microdensitometer (Model CS 900). The Rf values of galacturonic acid, galactose, arabinose, xylose, and rhamnose were 0.19, 0.51, 0.56, 0.73, and 0.84, respectively. Galacturonic acid constituted 95.4% of the total polysaccharide (Table 2).

Degree of esterification and intrinsic viscosity were examined by the methods of Gee et al.[6] and Owens et al.,[7] respectively. The degree of the esterification of the pectin was 70%. The degree of the polymerization seemed to be low, because the intrinsic viscosity was low (0.6 d*l*/g).

The pectin was dissolved in water to give a concentration of 0.2%. The pectin solution (2m*l*) was mixed with 0.1 M potassium tartrate buffer (2m*l*) with different pH (pH 2.5 to 4.5). The mixture was allowed to stand at room temperature for 48h and centrifuged at 30,000rpm for 30min. The pectin content in the supernatant was measured by the carbazole-$H_2SO_4$ method. About 14 % of the amount of the pectin added at pH 2.5 and 3% of it at pH 3.0 were precipitated, but the pectin was not precipitated at pH larger than 3.0 (Fig. 1a).

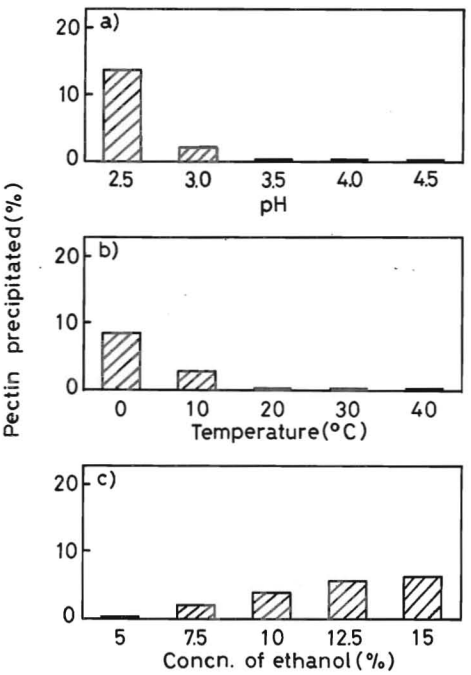


Fig. 1. Effects of pH, temperature, and ethanol on the precipitation of the pectin.

The pectin solution (2m*l*) was mixed with the pH 3.0 buffer (2m*l*) and allowed to stand at various temperatures for 48h, but no pectin was precipitated between 20 and 40°C (Fig. 1b).

The pectin solution was mixed with the pH 3.0 buffer containing various concentrations of ethanol. The other experimental

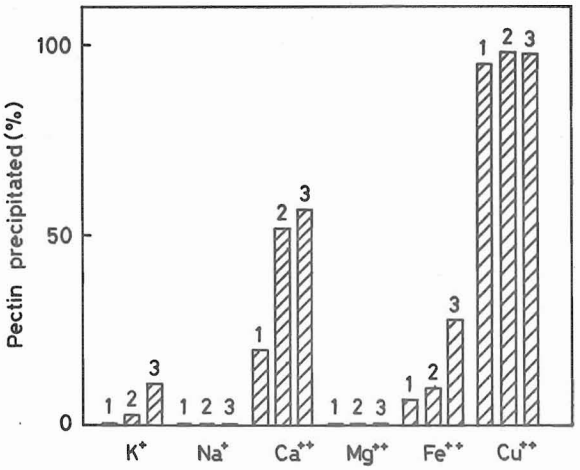


Fig. 2. Effects of various cations on the precipitation of the pectin.
The concentration of each cation was 5 mM (1), 10 mM (2), and 15 mM (3).

conditions were the same as described above. Although the amount of the precipitated pectin increased with an increase in the ethanol concentration, only 5% of the total pectin added was precipitated even when the concentration of ethanol was 15% (Fig. 1c). On the contrary, a large amount of pectin precipitate was produced by the addition of low concentrations of some cations (Fig. 2). The pectin solution (2 m*l*) was mixed with the pH 3.0 buffer containing various concentrations of a cation salt such as potassium tartrate, sodium tartrate, calcium tartrate, magnesium sulfate, copper sulfate, or iron sulfate. The concentration of each cation was from 5 mM to 15 mM. The cations such as $Cu^{++}$ $Ca^{++}$ and $Fe^{++}$ combined with the pectin and insoluble complexes occurred. However, the concentrations of these cations in the Koshu must or wine were usually below 0.1 mM. Therefore, the formation of precipitate due to binding of the pectin to these cations seemed improbable to occur. The concentration of $K^+$ in the wine was about 25 mM. The addition of $K^+$ at the concentration of 15 to 50 mM produced the precipitate corresponding to 9 to 11% of the original amount of the pectin.

The Koshu must (60 *l*) was dialized against running water for 24 h. The dialyzate (69.5 *l*) was centrifuged at 8,000 rpm for 30 min. To the supernatant, DEAE-Sephadex A-50 gels (69.5 g as dry weight) equilibrated with 1/100 M phosphate buffer (pH 6.5) were added. The mixture was adjusted to pH 6.5 with dilute NaOH and stirred at 4°C for 24 h. The mixture was filtered through a Toyo filter No. 2 with a Büchner funnel. The gels collected were washed with 5 *l* of the pH 6.5 phosphate buffer containing 2 M NaCl. The eluate was dialyzed against deionized water overnight. To the dialyzate, DEAE-Sephadex A-50 gels (8 g as dry weight) were added and stirred. The mixture was packed into a glass column, and the column was eluted with the pH 6.5 buffer containing 2 M NaCl. A protein concentration was measured at 280 nm. A portion of proteins was collected and dialyzed three times against a large amount of water. The dialyzate was lyophilized. The yield of the

must protein was 630mg by weight. All these procedures were carried out at 4℃.

The protein concentration of the lyophilisate was estimated from the sum of all amino acids found by amino acid analysis after hydrolysis. Amino acid analysis was carried out according to the method described previously.[8] The amino acid composition of the must protein was as follows : aspartic acid, 15.7% : threonine, 11.4% : serine, 8.7 % : glutamic acid, 8.2% ; proline, 5.6% ; glycine, 12.7% ; alanine, 9.9% ; valine, 4.9% ; isoleucine, 2.3%, leucine, 4.7% ; tyrosine, 2.5% ; phenylalanine, 6.5% ; histidine, 1.6% ; lysine, 3.0% ; and arginine, 2.2% (mole %). The amino acid composition was similar to that of the must protein obtained by precipitation with trichloroacetic acid.[8] The pectin (0.5g) was dissolved in 100m*l* of water. The must protein was dissolved in 1/100 M potassium tartrate buffer, pH 3.0, to give the final concentrations of 20 $\mu$g/m*l* to 100 $\mu$g/m*l*. The protein solution (0.5m*l*) was mixed with the mixture (3.5m*l*) of the pectin solution (0.8m*l*) and the pH 3.0 buffer (2.7m*l*). The sample solution was stored at room temperature for 48h, and centrifuged at 40,000rpm for 30min. The amount of the pectin precipitate increased with an increase in the amount of the protein (Fig. 3).

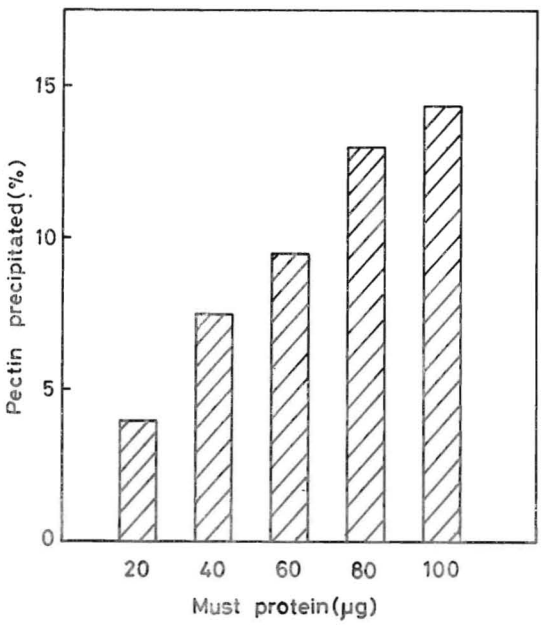


Fig. 3. Effects of Koshu must protein on the precipitation of the pectin.

Thus, the pectin was bound to the must protein to produce the insoluble complexes, but the phenols isolated from Koshu wine by the extraction method[9] and bovine serum albumin (fraction V, Sigma) gave no effect on the precipitation of the pectin.

## References

1) Bitter, T., Muir, H. M. : *Anal. Biochem.*, **4**, 330 (1962).
2) Hodge, J. E., Hofreiter, B. T. : *Methods in Carbohydrate Chemistry* (Whistler, R. L., Wolfrom, M. L.), Vol. 1, 388, Academic Press, New York and London (1962).
3) Singleton, V. L., Rossi, J. A., Jr. : *Amer. J. Enol. Vitic.* **16**, 144 (1965).
4) *Receuil des Methodes internationales d'Analyse des Vins,* 5th ed., Office International de Vigne et du Vin, Paris, 1978.
5) Bailey, R. W., Bourne, E. J. : *J. Chromatogr.*, **4**, 206 (1960).
6) Gee, M., Reeve, R. M., McCready, R. M. : *J. Agr. Food Chem.*, **7**, 34 (1959).
7) Owens, H. S., McCready, R. M., Shepherd, A. D., Schultz, E. L., Swenson, H. A., Miers, J. C., Ehrlandsen, R. F., Maclay, W. D. : *U. S. Dept. Agr., Bur. Agr. Ind. Chem.*, AIC 340 (1952).
8) Yokotsuka, K., Yoshii, M., Aihara, T., Kushida, T. : *J. Ferment. Technol.*, **55**, 510 (1977).
9) Yokotsuka, K., Takahashi Y., Yoshii, M., Kushida T. : *J. Ferment. Technol.*, **56**, 122 (1978).

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