# Tannin Extraction from Mangosteen Peel for Protein Precipitation in Wine

*Presented in the 3rd International Conference for Value Added Agricultural Products (3rd FerVAAP Conference)*

*Khanittha Moosophin[1,2]*, Tanyaporn Wetthaisong[1]*
*La-ong Seeratchakot[1], Wilasinee Kokluecha[1]*

## Abstract

The early ripe mangosteen was used for tannin extraction. The factors affecting tannin extraction such as size of the mangosteen peels, solvent types, temperature, extraction period and the ratio of mangosteen peel to solvent were studied. Extraction of the mangosteen peel sized 20/30 mesh with the mixture of water and 95% ethanol (1:1 v/v) at 80°C for 2 hours and the ratio of mangosteen peel to solvent of 1:10 (w/v) gave the highest yield of tannin. The crude extracted tannin was purified by adsorption chromatography on Sephadex LH-20. The recovery yield of the purified tannin was 27.48% of the total tannin. The chemical test results of the purified tannin showed that the condensed tannin was the predominant type of tannin in mangosteen peel. Since the condensed tannin can be applied to precipitate protein, the purified tannin obtained from the experiment was then used for protein precipitation in wine. The results from the consumer test showed that the wine clarity and the increasing of the wine's astringency were noticeable at 95% significance level.

**Keywords:** extraction, tannin, mangosteen, wine

[1] *Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand*
[2] *Fermentation Research Center for Value-Added Agricultural Products, Khon Kaen University, Khon Kaen 40002, Thailand*
* *Corresponding author, e-mail: khamoo@kku.ac.th*

# Introduction

Tannins are polyphenolic compounds found in the plant kingdom. Their main characteristic is the capability of binding and precipitating proteins. Tannins have molecular weights ranging from 500 to 3,000 (Viriwutthikorn, 1996). Tannins are usually divided into hydrolysable tannins and condensed tannins (proanthocyanidins). A hydrolysable tannin molecule contains a carbohydrate (usually D-glucose) at a center core. The hydrolysable tannins are readily hydrolyzed by enzymes as well as weak acids, into a sugar such as glucose, and a phenolcarboxylic acid such as gallic acid. In contrast, the condensed tannins are polymers of flavonoid units combined by carbon-carbon bonds resistant to enzymatic degradation. Upon acid treatment, condensed tannins not only decompose with the liberation of a small amount of anthocyanidins, but also progressively polymerize to yield amorphous phlobaphens or tannin reds (Strumeyer and Malin, 1975). The condensed tannins are more widely distributed in the higher plants than hydrolysable tannins. Tannins can be used in many industries such as tanning leather, ink manufacture, particle board, cosmetics and pharmaceuticals. Using tannin in beverage such as wines can enhance wine clarity because tannin can precipitate proteins in wine. Moreover, tannin as an astringent can increase wine's astringency.

At present, tannin can be extracted mainly from Mangrove cutch in Thailand. Unfortunately, the amounts of Mangrove cutch decrease dramatically whereas the demands of tannin are high. In 1991 Thailand imported tannin for 65,770,410 baht and tended to increase yearly (Wongsiri, 1993). To avoid using barks of many plants, new resources which are potential and have enough tannin are needed. Thailand has many agricultural wastes which are suitable to be used for tannin extraction. Surojanamethakul and Hiraga (1994) extracted tannin from banana peel. The recovery yield of tannin from one-time extraction was 81-85% of the total tannin with 2 hours soaking in 50% ethanol at 50$^{o}$C, and the ratio of banana peel to solvent was 1:30 or 1:40 w/v. Tannin from banana peel was found to be mainly condensed tannin. Since the banana peel has a low tannin content, industrial extraction is not economical. Mangosteen is another interesting source for tannin extraction. The office of agricultural economics, Thailand (2009) reported that in 2008, Thailand had an area of 396,325 rai of mangosteen plantation with the annual production of fresh mangosteen of 173,511 tons. The main locations of mangosteen farms are in the South and the East of Thailand. Consumers like to eat fresh mangosteen. About 30% of fresh mangosteens are eaten and the rest is the peel which is not edible and becomes garbage (Sangkhapaitoon et al., 2008). Theppoonpol (1995) reported that mangosteen peel consisted of 14.1% tannin. Therefore, the objectives of this research were to determine the optimum conditions for tannin extraction from the mangosteen peel. Furthermore, isolation, purification and some properties of extracted tannin were studied. Finally, the purified tannin obtained from the experiment was then applied in wine making since tannin can precipitate protein in wine and increase wine's clarity. Tannin also increases wine's astringency which will improve the taste of wine. In this experiment, Roselle, a low astringency fruit, was used for making wine.

# Materials and Methods

### *1. Optimum conditions for tannin extraction*

The early ripe mangosteen fruits were randomly bought from Bang Lum Poo market, Khon

Kaen, Thailand in July-September. The magosteen peel was dried in an oven at 50°C for 6 hours. The dried mangosteen peel was blended to small sizes by a blender and then was sieved through 20, 30 and 40 mesh. Two sizes of mangosteen peel (20/30 and 30/40 mesh) were used to determine the optimum conditions for tannin extraction. One-time extraction and the ratios of magosteen peel to solvent 1:10 and 1:30 (w/v) were studied. Solvent types were water, 95% ethanol-water (1:1 v/v) and 95% ethanol. Extraction periods were 1, 1.5 and 2 hours. Extraction temperatures were 30, 50 and 80°C. Each experiment was performed in triplicate.

One gram of mangosteen peel was added into a 250-ml Erlenmeyer flask and mixed with solvents at the selected ratio. The Erlenmeyer flask was incubated in a water bath at the selected temperature with the shaking speed of 150 rpm. The quantitative analysis of tannin was performed spectrophotometrically, using the spectrophotometer (Shimadzu, UV-1201). Standard solutions were formulated by Folin Dennis reagent and the calibration curve was developed at 760 nm (AOAC 1990).

### *2. Isolation and purification of tannin*

The optimum conditions from the previous step were used for tannin extraction. Thirty grams of mangosteen peel was extracted in three subsequent mixtures of 95% ethanol and water (1:1 v/v) with the ratio of 1:10 at 80°C for 2 hours. Insoluble materials which contained very little tannin were removed by filtration with a nylon filter and centrifuged at 3500 rpm for 15 minutes. The ethanol in the solution was removed by a rotary evaporator at 40°C. The remaining tannin was mixed with an equal volume of 1 mM acetate buffer at pH 4, and the remaining aqueous phase was extracted twice with an equal volume of ethyl acetate. The ethyl acetate fraction, which contained some amorphous solid materials, was discarded. The aqueous phase was evaporated to dryness by the rotary evaporator, redissolved in a minimum volume of 80:20 ethanol-water (v/v), and then chromatographed on a 2.5 cm x 30 cm column of Sephadex LH-20 (Pharmacia Fine Chemicals), which was previously equilibrated with absolute ethanol. The gel was washed repeatedly with absolute ethanol at the flow rate of 0.8 ml/min until the 280 nm absorbance of the washes reached a constant minimum value. The gel was then washed with 50:50 acetone-water (v/v) at the flow rate of 0.9 ml/min until the absorbance at 540 nm reached a constant minimum value. The 50% acetone fraction, which contained the tannin, was evaporated by the rotary evaporator to remove acetone, and the aqueous solution was extracted three times with an equal volume of liquefied phenol. The aqueous phase was washed with a small amount of diethyl ether to remove phenol, evaporated to dryness, and redissolved in a minimum volume of absolute ethanol. This material was chromatographed on Sephadex LH-20 with an absolute ethanol mobile phase at the flow rate of 0.9 ml/min, and monitored at 280 nm. When a stable base line was reached, the mobile phase was changed to 50% aqueous acetone, and monitored at 540 nm. The fractions containing tannin were pooled on the basis of the retention time. The portions were lyophilized to yield light brown fluffy powders.

### *3. Chemical property analysis of extracted tannin*

The extracted tannin, standard hydrolysable (tannic acid, Merck) and standard condensed tannins ((+)-catechin, Sigma-Aldrich) were assayed for tanning properties by their abilities to interact with ferric chloride, gelatin, lead acetate and bromine water. The amount of 0.25 g of the tannin samples were dissolved with distilled water and the final volumes

were adjusted to 50 ml. Maximum absorbances of the solutions were measured. Each 2 ml of the solution was mixed with 2-3 dropwises of 1% ferric chloride (Merck), 1 ml of 1% gelatin (Fluka), 1 ml of 1% lead acetate (Merck) and 1 ml of bromine water (Merck).

***4. Precipitation of proteins in Roselle wine by purified tannin***

Roselle juice was prepared by boiling 200 g of dried Roselle calyces with 2 l of water and sugar was added until the total soluble solids of 21°Brix was obtained. The fermentation was done by *Saccharomyces cerevisiae* strain Burgundy until 11% of alcohol and 5°Brix of total soluble solids were reached. The amount of 100 mg/l of potassium metabisulfite was added to stop the growth of the microorganisms. Roselle wines were precipitated with albumen (0.15% v/v) and albumen together with purified tannin which was obtained from the previous step. The amount of 0.1 g of purified tannin was used for 100 ml of Roselle wine. Consumer test was done to compare the clarity and astringency of wines. For the consumer test, 50 judges were recruited from students of the Department of Biotechnology, Khon Kaen University (Thailand). All data obtained from the consumer test were subjected to statistical analysis using one-way ANOVA. Significance of variations in the analyzed data was tested at 95% confidence limit.

## Results and discussion

***1. Optimum conditions for tannin extraction***

The factors affecting tannin extraction such as size of the mangosteen peels, solvent types, temperature and extraction period, were studied. Tables 1 and 2 present the abilities of solvent types for tannin extractions from mangosteen peel. The results showed that the abilities of solvents for tannin extraction from high to low were the mixtures of 95% ethanol and water (1:1 v/v), water and 95% ethanol. Increasing in the extraction temperature and time gave higher tannin except 95% ethanol. Smaller particle sizes of mangosteen peel resulted in better extraction due to their higher surface areas for interaction with solvents. The optimum conditions were the extraction of the mangosteen peel sized 20/30 mesh with the mixture of water and 95% ethanol (1:1 v/v) at 80°C for 2 hours, and the ratio of mangosteen peel to solvent of 1:10 (w/v) gave the highest yield of tannin (11.14%).

**Table 1.** Tannin extraction from 20/30 mesh mangosteen peel

| Temperature (°C) | The ratio of mangosteen to solvent | Time (hrs.) | Tannin (%) | | |
|---|---|---|---|---|---|
| | | | Water | 95% Ethanol : Water (1:1 v/v) | 95% Ethanol |
| 30 | 1:10 | 1 | 1.37 | 4.96 | 0.35 |
| | | 1.5 | 1.54 | 5.01 | 0.45 |
| | | 2 | 1.58 | 5.49 | 0.45 |
| | 1:30 | 1 | 0.67 | 2.33 | 0.23 |
| | | 1.5 | 0.73 | 2.72 | 0.36 |
| | | 2 | 0.74 | 3.12 | 0.37 |
| 50 | 1:10 | 1 | 2.19 | 8.73 | 0.45 |
| | | 1.5 | 2.22 | 9.44 | 0.47 |
| | | 2 | 2.51 | 9.76 | 0.56 |
| | 1:30 | 1 | 0.46 | 5.27 | 0.39 |
| | | 1.5 | 0.90 | 5.88 | 0.40 |
| | | 2 | 0.97 | 6.26 | 0.47 |
| 80 | 1:10 | 1 | 2.98 | 8.22 | n.d. |
| | | 1.5 | 3.65 | 9.38 | n.d. |
| | | 2 | 3.75 | 11.14 | n.d. |
| | 1:30 | 1 | 1.47 | 5.44 | n.d. |
| | | 1.5 | 1.93 | 5.80 | n.d. |
| | | 2 | 2.33 | 6.31 | n.d. |

n.d. = not detected

**Table 2.** Tannin extraction from 30/40 mesh mangosteen peel

| Temperature (°C) | The ratio of mangosteen to solvent | Time (hrs.) | Tannin (%) | | |
|---|---|---|---|---|---|
| | | | Water | 95% Ethanol : Water (1:1 v/v) | 95% Ethanol |
| 30 | 1:10 | 1 | 1.08 | 4.31 | 0.26 |
| | | 1.5 | 1.18 | 4.55 | 0.33 |
| | | 2 | 1.37 | 4.67 | 0.38 |
| | 1:30 | 1 | 0.47 | 2.27 | 0.27 |
| | | 1.5 | 0.53 | 2.54 | 0.32 |
| | | 2 | 0.56 | 3.58 | 0.37 |
| 50 | 1:10 | 1 | 1.29 | 5.64 | 0.26 |
| | | 1.5 | 1.36 | 6.43 | 0.30 |
| | | 2 | 1.43 | 5.74 | 0.30 |
| | 1:30 | 1 | 0.56 | 4.04 | 0.39 |
| | | 1.5 | 0.61 | 4.40 | 0.41 |
| | | 2 | 0.63 | 4.50 | 0.41 |
| 80 | 1:10 | 1 | 1.21 | 6.43 | n.d. |
| | | 1.5 | 1.25 | 6.84 | n.d. |
| | | 2 | 1.35 | 7.48 | n.d. |
| | 1:30 | 1 | 1.72 | 3.81 | n.d. |
| | | 1.5 | 1.77 | 4.48 | n.d. |
| | | 2 | 2.05 | 4.58 | n.d. |

n.d. = not detected

***2. Isolation and purification of tannin***

The optimum conditions from the previous step were used. Mangosteen peel sized 20/30 mesh was extracted with the mixture of 95% ethanol and water (1:1 v/v) at 80°C for 2 hours and the ratio of mangosteen peel to solvent of 1:10 (w/v). Non-tannin contaminants which must be removed from the tannin-containing extract include low molecular weight phenolics and pigments (Hagerman and Butler, 1980). Some of these components are extracted into ethyl acetate, and others can be removed by adsorption chromatography with Sephadex LH-20 (Strumeyer and Malin, 1975). In absolute ethanol, the gel has a high affinity for aromatic materials such as tannin (Asquith et al., 1983). When an absolute ethanol extract was applied to Sephadex LH-20, the non-tannins were removed by exhaustive washing with absolute ethanol. These non-tannin components were resolved into series of fractions visually distinguishable by color. In contrast, the tannins remained tightly adsorbed to the top of the column in absolute ethanol and could be observed as an immobile, yellowish brown band. Elution of the column with 50% aqueous acetone subsequently released the tannins. For tannin preparations, phenol is a suitable protein solvent to remove traces of protein contaminations. Thus, phenol fractionation was employed in the second step to reduce protein contamination of tannin (Hagerman and Butler, 1980). Excess phenol was removed by diethyl ether. The non-tannins were removed by adsorption chromatography with Sephadex LH-20. In order to get good separation, the flow rate of solvent should not exceed 1 ml/min. The tannins remained adsorbed to the top of the column in absolute ethanol and could be observed as an immobile, brown band. The tannins were released rapidly when the column was eluted with 50% aqueous acetone. These portions were lyophilized and yielded light brown fluffy powders containing 27.48% of total tannin.

***3. Chemical property analysis of extracted tannin***

Table 3 shows chemical property tests of purified tannin. The extracted tannin from mangosteen peel was characterized by its ability to interact with metal ions and precipitate proteins such as gelatin and metal salts such as lead acetate. Moreover, only the condensed tannins gave precipitate with bromine water. The chemical test results of the brownish purified tannin showed that the condensed tannin was the predominant type of tannin in mangosteen peel.

**Table 3.** Chemical property tests of purified tannin

| Tannin | Maximum wavelength (nm) | Test | | | |
|---|---|---|---|---|---|
| | | 1% $FeCl_3$ | 1% gelatin | 1% lead acetate | Bromine water |
| Condensed tannin | 277 | Green color and precipitate | precipitate | Reddish brown color and precipitate | Yellow color and precipitate |
| Hydrolysable tannin | 273 | Bluish black color and precipitate | precipitate | Dark red color and precipitate | No precipitation |
| Purified tannin | 279 | Greenish black color and precipitate | precipitate | Reddish brown color and precipitate | Yellow color and precipitate |

***4. Precipitation of proteins in Roselle wine by purified tannin***

The condensed tannin exhibited the ability to precipitate protein and metal ions (Surojanamethakul and Hiraga, 1994). The purified tannin obtained from the experiment was then used for protein precipitation in wine. Roselle wine was used due to its low astringency. Tannin-protein interactions are mainly based on hydrogen bond formation between the phenolic hydroxyl groups of the tannin and the carbonyl groups of the protein peptide bond. Ionic and covalent bondings also occur but play a minor role (Cannon, 1955; Calderon et al., 1968; Gustavson, 1954; Van Buren and Robinson, 1969). The interaction between tannin and protein can form formation of both soluble and insoluble complexes. Their relative proportion depends on the concentration and size of both molecules. In order to get precipitation, both the tannin and the protein must have the correct steric structure and molecular weight. The molecular weight of the tannin that causes protein precipitation appears to be around 1000-3000 (Joslyn and Goldstein, 1964).

Tannin also contributes to astringency. The dry or puckery sensation of astringency occurs from the cross-linking of the protein and glycoprotein in the mouth by tannins with a correspondingly reduced lubricant action. Low molecular weight tannins are apparently too small to create sufficiently effective cross-links and are therefore not noticeably astringent. Highly polymerized tannins are either too insoluble or too large to fit between suitably oriented protein molecules, and maximum astringency is most probably shown by tannins of intermediate size (Goldstein and Swain, 1963).

For wine fermentation, proteins are the main compositions of nitrogen compounds which are found in wines from fruits and play an important role for fermentation, clarity and stability of wine. In this experiment, Roselle was used for wine fermentation. Roselle contained protein of 6.26 g/100 g dry weight and low tannin (Danvirutai and Laopaiboon, 2005). Albumen was applied to precipitate protein in Roselle wine. Albumen contains albumin, soluble protein and has positive charge, and typically used as a fining agent for red wines. Albumin and other proteins in wine could be precipitated by interaction with tannin, contained in wine. Table 4 shows the average scores from consumer test data. The clarity and astringency of the wine precipitated with albumin and purified tannin had mean values of 1.98 and 1.94 respectively, while the wine precipitated with only albumin scored 1.50 and 1.58, respectively. The analysis by ANOVA showed that the wine precipitated with albumin and purified tannin did differ significantly from that precipitated with only albumin ($P < 0.05$) in clarity and astringency.

**Table 4.** Average scores from consumer test by 3 hedonic scaling

| Properties | Roselle wine precipitated with albumin | Roselle wine precipitated with albumin and purified tannin |
|---|---|---|
| Clarity | $1.50^{a}$ | $1.98^{b}$ |
| Astringency | $1.58^{a}$ | $1.94^{b}$ |

Note: means followed with different letters are significantly different at $P < 0.5$

## Conclusion

Tannin was extracted from mangosteen peel. The optimum conditions were the extraction of the mangosteen peel sized 20/30 mesh with the mixture of water and 95% ethanol (1:1 v/v) at 80°C for 2 hours and the ratio of mangosteen peel to solvent of 1:10 (w/v) gave the highest content of tannin. The crude extracted tannin was purified and the chemical test results of the purified tannin showed that the condensed tannin was the predominant type of tannin in mangosteen peel. The purified tannin was then applied for protein precipitation in wine. The results from the consumer test showed the wine clarity and the increasing of the wine's astringency were noticeable at 95% significance level. It could be concluded that the condensed tannin extracted from mangosteen peel exhibits the ability to precipitate protein in wine and also increases the wine's astringency.

## Acknowledgements

The authors gratefully thank the financial support by Khon Kaen University, Khon Kaen, Thailand.

## References

AOAC. 1990. **Official Methods of Analysis.** 15th edition. Arlington : Association of Official Analytical Chemists.

Asquith, T.N., Izuno, C.C. and Butler, L.G. 1983. Characterization of the condensed tannin (proanthocyanidin) from a group II sorghum. **Journal of Agricultural and Food Chemistry.** 31(6): 1299-1303.

Cannon, C.G. 1955. The interactions and structure of the -CONH- group in amides and polyamides. **Mikrochimica Acta.** 43(2-3): 555-588.

Calderon, P., Van Buren, J. and Robinson, W.B. 1968. Factors influencing the formation of precipitates and hazes by gelatin and condensed and hydrolyzable tannins. **Journal of Agricultural and Food Chemistry.** 16(3): 479-482.

Goldstein, J.L. and Swain, T. 1963. Changes in tannins in ripening fruits. **Phytochemistry.** 2: 371-383.

Gustavson, K.H. 1954. Interaction of vegeable tannins with polyamides as proof of the dominant function of the peptide bond of collagen for its binding of tannins. **Journal of Polymer Science.** 12: 317-324

Hagerman, A.E. and Butler, L.G. 1980. Condensed tannin purification and characterization of tannin-associated proteins. **Journal of Agricultural and Food Chemistry.** 28(5): 947-952.

Joslyn, M.A. and Goldstein, J.L. 1964. Astringency of fruits and fruit products in relation to phenolic content. **Advance in Food Research.** 13: 179-217.

Office of agricultural economics, Thailand. 2009. **Mangosteen,** [online] [cite 10 July 2009] Available from: http://www.oae.go.th/download/document/mangostrean51.pdf.

Theppoonpol, M. 1995. Utilization of mangosteen peel to improve soil fertility. **Soil and Fertilizer Journal.** 17: 3-5.

Sangkhapaitoon, P., Sangkhapaitoon, P. and Kummee, S. 2008. Antibacterial Activity of Mangosteen Hull Extracts Against *Staphylococcus aureus* from Infected Skin

Wound in Swine. [online] [cite 10 July 2009] Available from: http://rdi.rmutsv.ac.th/ebook/Content_Agri/400.pdf

Strumeyer, D.H. and Malin, M.J. 1975. Condensed tannins in grain Sorghum: isolation, fractionation, and characterization. **Journal of Agricultural and Food Chemistry.** 23(5): 909-914.

Surojanamethakul, V. & Hiraga, C. 1994. Extraction of tannin from banana peel. **Kasetsart Journal: Natural Science.** 28: 578-586.

Danvirutai, P. and Laopatboon, P. 2005. **Fruit and Rice Wines: Confidence in Winemaking,** Khon Kaen : Klungnanawittaya Press.

Wongsiri, W. 1993. **Extraction of Tannin from Rambutan Peel.** Master Thesis, Department of Chemical Technology, Chulalongkorn University, Bangkok.

Van Buren, J.P. and Robinson, W.B. 1969. Formation of complexes between protein and tannic acid. **Journal of Agricultural and Food Chemistry.** 17(4): 772-777.

Viriwutthikorn, W. 1996. The importance of tannin for food industries. **Journal of Food.** 26(3): 157-167.