JARQ 35(3), 185 – 188 (2001) http://www.jircas.affrc.go.jp

Development of a Simple Preparation Method for Tea-Seed Saponins and Investigation on Their Antiyeast Activity

Yuji YAMAUCHI[1], Keiko AZUMA[2], Minoru TOMITA[3], Hideki HORIE[4] and Katsunori KOHATA[4]*

[1] Graduate School of Pharmaceutical Sciences, Osaka University (1–6 Yamadaoka, Suita, Osaka 565–0871, Japan)
[2] National Institute of Vegetable and Tea Science (360 Ano, Mie 514–2392, Japan)
[3] Tokushima Prefectural Industrial Technology Center (11–2 Saika, Tokushima 770–8021, Japan)
[4] National Institute of Vegetable and Tea Science (2769 Kanaya, Shizuoka 428–8501, Japan)

Abstract

A simple preparation method was developed for tea-seed saponins (TSS) with improved quality. This method was applied to the preparation of TSS from 14 tea-seed cultivars. The yield of TSS was in the range of 10 ~ 13% per dry weight and not appreciably different among the cultivars. In addition, the antimicrobial activity of TSS against 6 yeast species was investigated. TSS showed a relatively high growth inhibitory activity against all the yeasts examined and their minimal inhibitory concentration (MIC) values varied from 0.08 to 1.20 mg/mL.

Discipline: Tea industry
Additional key words: tea-seed cultivars, growth inhibitory activity, minimal inhibitory concentration

Introduction

Saponins which are natural surfactants exhibit a variety of biological activities. As for the application of saponins, it is essential that sufficient amounts of plant resources be available, and that the content of saponins be high. Furthermore, the plants must have a long history of human use.

It has been recognized that tea-seed saponins (TSS) account for more than 10% per dry weight of tea seed[11], and that they display various physiological functions such as anti-expectorant[7] and anti-inflammatory[6] properties, etc.[9,10]. Recently we have reported the control effect of TSS against insect pests and mites[2]. Until now, TSS had not been efficiently utilized in spite of their various physiological functions, mainly due to the long and laborious preparation methods available[1,4], and because the biological activities of TSS were found to be relatively low, compared with those of chemical compounds. Nevertheless, TSS as other natural products could be utilized as biological agents since they are less polluting to the environment than their chemical counterparts.

In this report, the development of a simple and highly reproducible method for the preparation of TSS with improved quality is described. The method was applied to the preparation of TSS from 14 tea-seed cultivars. Furthermore, the antimicrobial activity of TSS against 6 yeast species was also investigated for the effective utilization of TSS. Although the antimicrobial activity of saponins is well documented[5,6], that of TSS has not been investigated sufficiently.

Preparation of TSS with improved quality

Tea seeds of cv. Yabukita were collected in a tea field of National Institute of Vegetable and Tea Science (Shizuoka Prefecture). After removal of the seed coat and skin, tea seeds (250 g) were ground using a mortar and pestle and dried under reduced pressure at 40ºC to obtain a powder (129.09 g). This powder was defatted with n-hexane (500 mL × 2) to obtain tea-seed oils (oil fraction: 27.17 g, 21.05%), and then extracted with 80% (v/v) methanol (500 mL × 2). The resulting extract was evaporated to dryness under reduced pressure at below 40ºC to yield crude TSS as a light brown solid (crude TSS fraction: 36.51 g, 28.28%). This solid (1.67 g) was dissolved in 40% methanol (5 mL, v/v) to obtain a LPLC

*Corresponding author: fax+81–547–46–2169, e-mail kks914@tea.affrc.go.jp
Received 27 November 2000, accepted 5 February 2001.

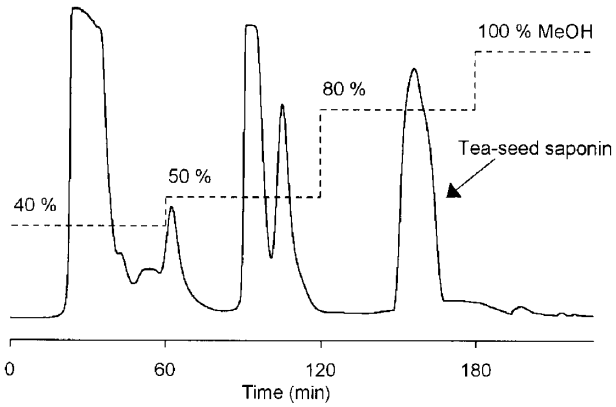

Fig. 1. LPLC chromatogram of crude tea-seed saponins (TSS) from cv. Yabukita extracted with 80% methanol

Column: Lobar pre-pack RP-18 (MERCK, 25 mmϕ × 31 cm), Detection: 250 nm, Flow rate: 3 mL/min, Injection: 5 mL of 40% methanol containing 1.67 g of sample, Mobile phase: stepwise gradient system from 40 to 100% methanol.

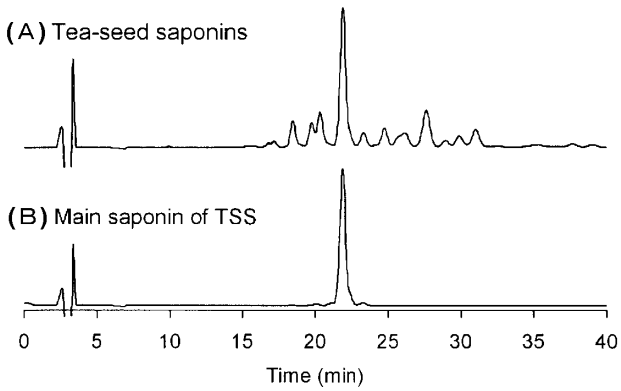

Fig. 2. HPLC chromatograms

(A) Tea-seed saponins (TSS) from cv. Yabukita, (B) Main peak saponin (TS-1) of TSS. Column: YMC-Pack ODS-AM (4.6 mmϕ × 25 cm), Detection: 225 nm, Flow rate: 1 mL/min, Injection: 20 µL of 3 mg per 1 mL 80% methanol, Mobile phase: acetonitrile/tetrahydrofuran/water/trifluoroacetic acid (40/5/70/0.1, v/v).

sample, which was injected into a Lobar pre-pack column RP-18 (MERCK, 25 mmϕ × 31 cm). The composition of the mobile phase was changed step by step from 40 to 100% methanol, as shown in Fig. 1. The flow rate was 3 mL/min. The fractions containing TSS were collected by monitoring at 250 nm with a UV detector and evaporated to dryness at below 40ºC to yield a white solid (0.77 g, 46.1%). The total yield of TSS based on the starting tea-seed powder was 13.1%. TSS obtained here were clearly isolated from other polar compounds (sugars, caffeine, etc.) and consisted of a mixture of more than 10 analogues (Fig. 2-A).

TSS were prepared from various tea-seed cultivars according to the method described above. Table 1 shows the yield of the oil fraction, crude TSS fraction and TSS. Although the yield of the oil fraction varied among all the cultivars examined, that of the crude TSS fraction and TSS did not change appreciably. Furthermore, similar HPLC chromatograms were obtained for all the samples, suggesting that TSS with a good quality could be obtained at a high yield regardless of the tea-seed cultivars. This method could be suitable for large-scale and routine preparation of TSS due to its simplicity and high reproducibility.

Isolation of a main tea-seed saponin (TS-1)

In the next step, TSS (155 mg) were dissolved in 1 mL of 80% methanol and a 50 µL aliquot was injected into a semi-micro-preparative column (YMC-Pack C8, 10 mmϕ × 30 cm). A mixture of acetonitrile / tetrahydrofuran / water / acetic acid in the proportion of 38 / 5 /70 / 0.1 (v/v) was used as a mobile phase and was flowed isocratically at a rate of 4.0 mL/min. The fractions containing the main saponin peak were collected by monitoring at 225 nm and evaporated to dryness under reduced pressure at below 40ºC. This operation was repeated 20 more times to yield 57.3 mg (37.0%) of a white solid, denoted

Table 1. Yield of tea-seed oil fraction, crude TSS fraction and TSS prepared from 14 tea-seed cultivars

Cultivar	Yield (%)		
	Oil	Crude TSS [a)]	TSS
For Gyokuro			
Ujihikari	11.09	35.10	11.32
Komakage	23.26	29.15	11.33
Samidori	11.60	29.53	10.55
For Sencha			
Yabukita	21.17	28.28	13.10
Sayamakaori	9.84	34.10	10.82
Shunmei	25.38	29.35	10.63
Takachiho	25.88	31.54	10.19
Meiryoku	24.90	33.14	10.42
For Oolong tea			
Seishintaipan	20.28	37.58	10.72
Obauron	14.96	36.37	11.27
For Black tea			
Inzatsu131	10.95	32.58	10.15
Hatsumomiji	23.97	31.91	10.37
Benihikari	24.76	31.46	12.52
Benihomare	26.60	36.30	12.14

a): Tea-seed saponins.

Note: Crude TSS fraction obtained from tea-seed powder by removal of oils followed by extraction with 80% methanol and evaporation.

Table 2. Antimicrobial activity of TSS against 6 yeast species

Yeast	MIC[a] (mg/mL)	
	TSS[b]	TS-1[c]
Debaryomyces hansenii (MAFF 113853)	1.20	
Hansenula anomala (MAFF 113717)	0.08	0.08
Saccharomyces cerevisiae (MAFF 113066)	0.30	0.15
Torulaspora delbrueckii (MAFF 113811)	0.15	
Zygosaccharomyces rouxii (MAFF 113447)	0.15	
Candida tropicalis (MAFF 114040)	0.30	

a): Minimal inhibitory concentration,
b): Tea-seed saponins from cv. Yabukita,
c): Main saponin of TSS.

here as TS-1. The total yield based on the starting tea-seed powder was 4.82%. TS-1 with a high purity was obtained (Fig. 2-B) and was assigned to the known theasaponin E_1[3] based on NMR and FAB-MS data. The chemical structure of tea sapogenins and sapogenols had already been published[13-15]. Sapogenins and sapogenols, aglycons of saponins, are relatively easy to prepare and characterize. However, direct isolation of individual saponins from TSS is difficult due to their surface-active properties and to the similarity in their chemical structure. This problem could be solved using the method we developed. Isolation and structural studies of other TS-1 analogues are currently in progress.

Antimicrobial activity of TSS

The physiological properties of TSS were elucidated by analyzing the antimicrobial activity against 6 yeast species (Table 2). YM broth containing 0.3% yeast extract, 0.3% malt extract, 0.5% peptone and 1.0% glucose was used as culture medium. TSS sample solution was prepared by dissolving TSS from cv.Yabukita into sterilized 50% ethanol (v/v). The mixture of YM broth (2.45 mL), yeast suspension (10 µL) and TSS sample solution (50 µL) was cultured by shaking at 25ºC for 24 h. The antiyeast activity was determined by visual observation of the turbidity of the culture. The minimal concentration, at which the turbidity did not change, was determined as the minimal inhibitory concentration (MIC). The culture time was 65 h for *Zygosaccharomyces rouxii*. It was evident that TSS exhibited a relatively high growth inhibitory activity against all the yeasts examined and the MIC values were in the range of 0.08 ~ 1.20 mg/mL (Table 2). The antiyeast activity of TS-1 against *Hansenula anomala* and *Saccharomyces cerevisiae* was almost the same as that of TSS, suggesting that the antiyeast activity of TSS might be attributed to that of TS-1. On the other hand, a low activity was found against other common fungi (data not shown).

Table 3. Antiyeast activity of TSS prepared from 14 tea-seed cultivars against *Zygosaccharomyces rouxii*

Cultivar	Concentration of TSS[a] (µg/mL)		
	5	10	20
For Gyokuro			
Ujihikari	–	±	+
Komakage	–	–	+
Samidori	–	±	+
For Sencha			
Yabukita	–	–	+
Sayamakaori	–	–	+
Shunmei	–	–	+
Takachiho	–	–	+
Meiryoku	–	–	+
For Oolong tea			
Seishintaipan	–	–	+
Obauron	–	–	+
For Black tea			
Inzatsu131	–	–	+
Hatsumomiji	–	–	+
Benihikari	–	–	+
Benihomare	–	–	+
TS-1[b]	–	–	+

a): Tea-seed saponins, b): Main saponin of TSS.
– : No inhibition, + : Inhibition.

In addition, we investigated the antimicrobial activity of TSS from 14 tea-seed cultivars against a halo-tolerant yeast, *Zygosaccharomyces rouxii* (ATCC 52698) which is a film-forming yeast damaging “soy sauce” and “miso”, oriental fermented seasonings. The procedure of Tomita[8] was slightly modified and adopted to determine the antiyeast activity. All the cultivars appeared to exhibit almost the same antiyeast activity with MIC values of 20 µg/mL (Table 3). The activity did not change appreciably among all the cultivars. A similar observation was made for TSS yield. The mechanism of TSS activity against yeasts remains to be elucidated.

Conclusion

We have already reported that the preparation method for TSS could be applied to the preparation of tea-leaf saponins with slight modifications[12] of the mobile phase system of LPLC. Since it has become easier to prepare tea-seed and tea-leaf saponins on a large scale, studies on tea saponins and their efficient utilization as an anti-food-deteriorating agent may be conducted in the near future.

References

1) De Silva, U. L. L. & Roberts, G.R. (1972): Products from tea seeds. 2-Extraction and properties of saponins. *Tea Q*., **43**, 91–94.
2) Kawai, A. et al. (1999): Control effect of tea seed saponins against insect pests and mites. *Tea Res. J*., **87**, 7–12 (1999) [In Japanese].
3) Kitagawa, I. (1992): Chemical investigation of naturally occurring drug materials. Elucidation of scientific basis for traditional medicines and exploitation of new naturally occurring drugs. *J. Pharm. Soc. Jpn*., **112**, 1–41 [In Japanese].
4) Mizuno, T. (1968): Studies on the carbohydrates of tea-plant. Part XII. The components sugars of theasaponin from the tea seed. *J. Agric. Chem. Soc. Jpn*., **42**,491–501 [In Japanese].
5) Murakami, F. (1996): Antimicrobe of yucca saponin and its utilization. *Food Process*., **31**, 13–16 [In Japanese].
6) Sagesaka, Y. et al. (1996): Antimicrobial and anti-inflammatory actions of tea-leaf saponin. *J. Pharm. Soc. Jpn*., **116**, 238–243 [In Japanese].
7) Tamura, S. (1956): Expectorant effect of saponin in seed of theaceous plants. *J. Pharm. Soc. Jpn*., **76**, 144–146 [In Japanese].
8) Tomita, M. et al. (1997): Physiological characteristics of a film-forming strain of *Zygosacharomyces rouxii*, and its cellular fatty acids synthesis. *Biosci. Biotech. Biochem*., **61**, 51–55.
9) Tsukamoto, S. et al. (1993): Effects of seed saponins of *Thea sinensis L*. (ryokucha saponin) on alcohol absorption and metabolism. *Alcohol & Alcohol.,* **28**, 687–692.
10) Wickremasinghe, R. L. et al. (1974): Effect of ethephon, pure tea seed saponin, and partially purified tea root saponin on the growth of tea in the nursery and the field. *Tea Q*., **44**, 132–143.
11) Xingtu, F. et al. (1990): Main chemical compounds and their content changes in tea fruit as it's ripening. *J. Tea Sci*., **10**, 49–58.
12) Yamauchi, Y. et al. (1998): Development of simple preparation method for tea saponins of improved quality. *Tea Res. J*., **87**, 120–121 [In Japanese].
13) Yoshioka, I. et al. (1970): Saponin and sapogenol. I. Seeds sapogenols of *Thea sinensis L*. (1) Barringtogenol C (=Theasapogenol B). *Chem. Pharm. Bull*., **18**, 1610–1620.
14) Yoshioka, I. et al. (1970): Saponin and sapogenol. I. Seeds sapogenols of *Thea sinensis L*. (2) Theasapogenol A. *Chem. Pharm. Bull*., **18**, 1621–1632.
15) Yoshioka, I. et al. (1971): Saponin and sapogenol. I. Seeds sapogenols of *Thea sinensis L*. (3) Theasapogenol E and minor sapogenols. *Chem. Pharm. Bull*., **19**, 1186–1199.