CHEMICAL EVALUATION OF GINSENG EXTRACTS: SEASONAL VARIATION OF SAPONINS AND SUCROSE IN CULTIVATED GINSENG ROOTS

S.K. KIM¹, I.Sakamoto, K. Morimoto², M. Sakata, K. Yamasaki and Osamu Tanaka³

¹Samnam Chemical Laboratories, Seoul, Korea ²Hiroshima Prefectural Institute of Public Health, Hiroshima, ³Institute of Pharmaceutical Sciences, Hiroshima University, Hiroshima, Japan

In connection with production of Ginseng extracts with high contents of the active principles, the seasonal variations of yields of the extracts and of contents of carbohydrates and saponins in the extracts have been investigated for fresh roots of *Panax ginseng* cultivated in Kumsan, Korea for four years.

The roots were harvested twice a month and the fresh whole roots without removal of side roots were extracted with methanol. Fig. 1 shows the seasonal variation of yields of methanolic extract calculated from the dry weight of the roots. The yields evidently decreased in April, the germination-season and were 20% or less until November. The yields increased evidently to about 40% in the beginning of December, somewhat decreased in January through February and then increased

again to about 40% in March.

The ¹³C-NMR spectrum of the extract in D₂O is shown in Fig. 2, indicating the high content of sucrose in it. We have developed the rapid quantitative analysis of sucrose in the extract by means of the peak height determination of each carbon signal using dioxan as an internal standard. The results were found to be in good accordance with those by the procedures such as the high performance liquid chromatography. As indicated in Fig. 3, the contents of sucrose in the extracts were between 60-80 % from December to the beginning of April (in winter) and remarkably decreased at the end of April, being 20-40% until October. This indicates that the high yields of the methanolic extracts in winter are mainly due to the significant increase of sucrose in the roots.

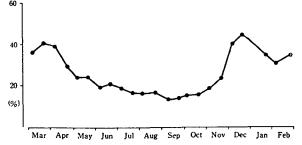


Fig. 1. Seasonal variation of yield (%) of MeOH Ext. from fresh roots of Panax ginseng, cultivated at Kumsan, Korea (4 Years)

(Calculated from dry weight of Roots)

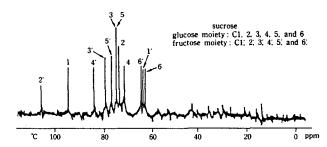


Fig. 2. ¹³C-NMR spectrum of MeOH Ext. of Panax ginseng in D₂O (25.15 MHz)

(Fresh roots harvested in August)

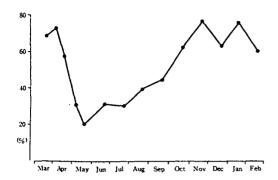


Fig. 3. Seasonal variation of sucrose content (%) in MeOH Ext. of fresh roots of Panax ginseng cultivated at Kumsan, Korea (4 Years)

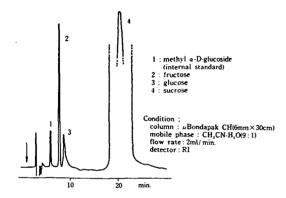


Fig. 4. HPLC of MeOH Ext. of fresh roots of Panax ginseng cultivated at Kumsan, Korea (4 years)
(Harvested on June/25)

Content of monosacchrides; glucose and fructose in the extracts were determined by the high performance liquid chromatography using methyl α -D-glucoside as an internal standard (see Fig.4). As shown in Table I, it was demonstrated that both glucose and fructose evidently increased just at the germination time (April) and decreased in summer. But anyway, relative contents of free monosaccharides; glucose and fructose to that of sucrose were extremely low, being negligible for the seasonal variation of the yield of the total extract.

Table 1. Seasonal variation of glucose and fructose contents (%) in MeOH Ext. of fresh roots of Panax ginseng Cultivated at Kumsan, Korea (4 years)

| | Mar./20 | June/20 | Sept./20 | Dec./20 | Jan./20 |
|----------|---------|---------|----------|---------|---------|
| Glucose | 2.2 | 0.5 | 0.1 | 0.9 | 0.5 |
| | (0.9) | (0.1) | (0.01) | (0.4) | (0.2) |
| Fructose | 1.6 | 1.1 | 0.2 | 0.6 | 0.4 |
| | (0.6) | (0.2) | (0.02) | (0.3) | (0.1) |

(): Contents (%) Calculated from dry weight of roots

A variety of biological activities of Ginseng saponins have been reported. The contents of the dammarane-saponins; 20S-protopanaxadiol group and 20S-protopanaxatriol group, in the fresh roots were determined by Sakamoto's procedure, gas liquid chromatography as trimethylsilyl ethers of panaxadiol and panaxatriol, being summarized in Fig. 5. The contents of both saponin-groups decreased at germination time and then increased, being total about 4%(calculated from the dry weight) until August. The saponin-content in winter was found to be low. Further, the contents of the individual majorsaponins; ginsenosides-Rb₁, -Rb₂, -Rc, -Rd, -Re and -Rg, were analyzed by the dual wave length thin layer chromatograph densitometry developed by Sanada, et al. As shown in Fig. 6, it was re-

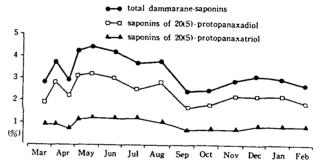


Fig. 5. Seasonal variation of ginseng-saponin contents (%) in fresh roots of Panax ginseng cultivated at Kumsan, Korea (4 Years)

(Calculated from dry weight of roots)

Total dammarane-saponins

Saponins of 20(S)-protopanaxadiol

A → Saponins of 20(S)-protopanaxatriol

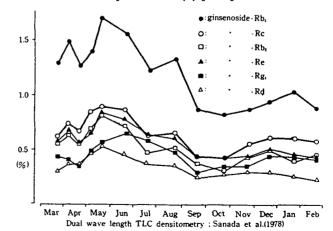


Fig. 6. Seasonal variation of contents (%) of individual Saponins in MeOH Ext. of fresh roots of Panax ginseng cultivated at Kumsan, Korea (4 years)

vealed that each major dammarane-saponins showed the similar seasonal variation of the content.

The present results are summarized in Fig. 7. It should be notable that in the Ginseng extracts of the roots harvested in November through April, the saponins and probably other active principles are evidently diluted with increased sucrose, though the yields of the extracts appearently very high in winter. Accordingly, the best season of harvest of the roots for production of the high quality extract as a medicine must be summer,

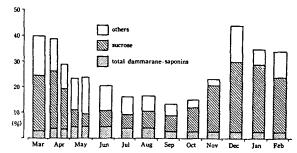


Fig. 7. Seasonal variation of contents (%) of constituents in MeOH Ext. of fresh roots of *Panax ginseng* cultivated at Kumsan, Korea (4 years) (Calculated from dry weight of roots)

Table 11. Contents(%) of constituents in methanolic extract of roots of Panax ginseng C.A. Meyer.

(): yields(%) caliculated from dry weight of roots

| Sample | MeOH- extract | ET ₂ O soluble saponins | | | sucrose | | amino acids | |
|--------|------------------|------------------------------------|--------|---------------------|---------|---------|---------------------|-----------|
| | | fraction | diolc) | triol ^{d)} | HPLC | 13C NMR | total ^{e)} | free form |
| Aa) | | 11.2 | 22.0 | 8.2 | 25.7 | 27.6 | 7.6 | 3.5 |
| | (17.3) | (1.9) | (3.8) | (1.4) | (4.4) | (4.7) | (1.3) | (0.6) |
| Вы | , , | 3.7 | 6.5 | 1.4 | 59.2 | 63.3 | 5.9 | 4.0 |
| | (43.6) | (1.6) | (2.8) | (0.6) | (25.8) | (27.6) | (2.6) | (1.7) |

- a) fresh roots harvested in August. b) roots^{a)} treated at 2° ± 1° for 30 days.
- c) saponins of 20 (S)-protopanaxadiol. d) saponins of 20(S)-protopanaxatriol.
- e) amino acids after hydrolysis with 6N HCl at 110° for 15 hr (free form + combined form)

from June to September as has been made customarily.

As already reported, it has been observed that treatment of the fresh roots harvested in summer at 2°C for one month resulted in the remarkable increase of yields of the extracts; from 17.3% to 43.6%. As shown in Table II, this increase of the extracts is also mainly due to the increase of sucrose¹. (The formation of sucrose from starch by treatment at low temperature was already observed for potato².) In this case, saponins, ether-soluble materials, amino acids, peptides, and probably other constituents in the extracts are also diluted with the increased sucrose just like the case of the extracts prepared from the roots harvested in winter. It follows that the fresh roots harvested in summer should not be stored in a cold room for a long time before the extraction or preparation of white- or red-Ginseng.

Acknowledgement

We are very much grateful to prof. Jae Hoon

Kim, Natural Products Research Institute, Seoul National University and prof. II Hyuk Kim, College of pharmacy, Chung-Ang University for their valuable advice and encouragement. This work was supported by the International Joint Research Program of Japan Society for Promotion of Science, to whom thanks are due.

Chairman: Now the time is open to discussion.

Proctor: Sir, did you make any study of the carbohydrate contents of the leaves during the studies?

Tanaka: Well, I have studied only on the saponins of the leaves of panax species but I am sorry I have not studied on the carbohydrate content in the leaves. I am going to do that. But anyway, the content of the carbohydrate in the leaves are very low comparing with the roots.

Staba: I enjoyed your paper very much. I have one short question, and that is how old were the plants that you studied and do you think it would make a difference of the roots were younger or older?

Tanaka: We used 4 year age roots. I have not studied on the case of one year or two year age roots. I'll have to do that.

Questioner (from Japan): Can you tell me the difference in the yield of extract content of ginseng roots cultivated in different area.

Tanaka: I think the yield of extract content of these ginseng must be very similar throughout Korea and Japan. But any way I have only studied on the ginseng roots cultivated in Keum San area of Korea.

References

- S.K.Kim, C. Hiyama, K.Yanasaki, R.Hiraoka,
 O. Tanaka, J.H.Kim and I.H.Kim; Shoyaku-gaku Zasshi, 33, 245 (1979).
- 2. B.Arreguin-Lozano and J.Bonner, Plant Physiol., 24, 720 (1949).