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Development of Quantitative Extraction Method of Amygdalin without Enzymatic Hydrolysis from Tōnin(Persicae Semen) by High Performance Liquid Chromatography

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Tōnin(Persicae Semen) is the herb medicine that contains amygdalin as a major ingredient. Amygdalin in water is decomposed into benzaldehyde, HCN, and glucose by emulsin, a hydrolysis enzyme in tōnin. A useful and practical method for the optimum extraction condition of amygdalin without enzymatic hydrolysis is required. The extraction yield of amygdalin of natural formula tō nin was 0.1% from crude powders, 1.4% from small pieces, 3.5% from half pieces and 2.4% from whole pieces. The extraction yield of amygdalin of outer shell-eliminated tō nin was 0.3% from crude powders, 1.4% from small pieces, and 3.5% from half pieces and whole pieces respectively. The extraction yield of amygdalin was most high when using the size larger than half.

Key words: Tōnin, Persicae Semen, Amygdalin, Emulsin, Enzymatic hydrolysis, Extraction yield

INTRODUCTION

Tōnin(Persicae Semen) is the herb medicine that contains amygdalin as a major ingredient. It has been generally used as a lubricant or an anti-stasis agent in traditional oriental medicine (Zhu, 1998). Recently, it has been reported that amygdalin kills cancer cells selectively at the tumor site without systemic toxicity, the problem of general chemical agents (Syrigos *et al.*, 1998).

In the formulae of oriental medicine, the crude powder of natural formula tōnin is extracted with water. However, it is uncertain whether the tōnin from water decoction can work as an anti-tumor agent, because amygdalin in water is decomposed into benzaldehyde, HCN, and glucose by emulsin, a hydrolysis enzyme in tōnin (Heisman *et al.*, 1967). Therefore, a useful and practical method for the higher extraction yield of amygdalin in water is required.

It has been known that extraction yield of amygdalin could be obtained higher when tōnin was used as small pieces rather than powders, but it could not reach quantitative extraction using its method (Akahori *et al.*, 1983).

In this study, we attempted to improve the optimum extraction condition for maximum extraction yield of amygdalin from tōnin. In order to obtain the quantitative extraction of amygdalin from tōnin, several conditions were extensively studied including the following factors: such as the cutting size of tōnin, the existence of seed coat, the solvent for extraction, and extraction time. Hydrolysis mechanism from emulsin has additionally investigated.

MATERIALS AND METHODS

Materials

Amygdalin was obtained from Tokyo Kasei Chemical Co. (Tokyo, Japan). Methanol was purchased from Merck (Darmstadt, German). The other reagents and solvents used were of guaranteed or analytical grade. Distilled water was further purified by a water purification system(Pure power III, Taiwan).

Apparatus and chromatographic conditions

The HPLC system consisted of a M930 pump (Young Lin, Kyunggi, Korea), a M720 UV detector(Young Lin,

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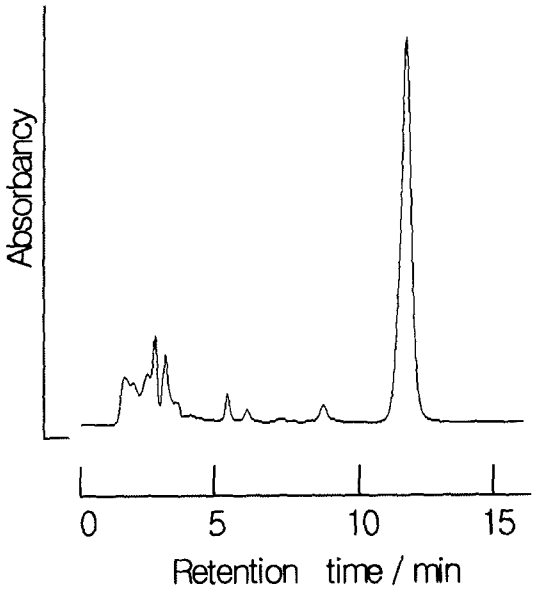

Fig. 1. Reversed-phase chromatogram of amygdalin from tōnin

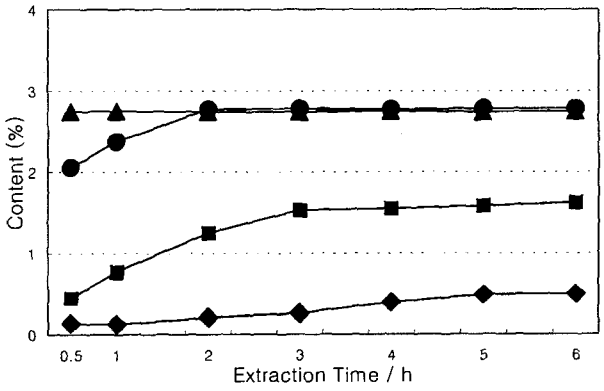

Fig. 2. Extraction yield of amygdalin according to cutting size of natural fomula tōnin with methanol.
▲ :crude powders, ● :small pieces, ■ :half pieces, □:whole pieces

Kyunggi, Korea) set at 214 nm. The column was a Capcell Pak C18, Type UG120(250 mm × 4.6 mm, Shiseido, Japan) at 35°C contained in a CTS30 column oven(Young Lin, Kyunggi, Korea). Mobile phase was 25% methanol-water at 1 mL min^{-1}.

Calibration and linearity

A calibration curve was constructed using six different concentrations of standard solution containing 30, 60, 90, 120 and 150 μg mL^{-1} of amygdalin. Peak-area was calculated for the calibration plot.

Extraction procedure

Tōnin was prepared in the form of crude powders, small pieces, half pieces and whole pieces. Each sample 2 g was extracted under reflux with 50 mL of methanol or distilled water for 0.5, 1, 2, 3, 4, 5 and 6 h. Each methanol extract was evaporated under low pressure and suspended with distilled water. Nonpolar substances were removed by treating with n-hexane over three times. The aqueous layers were filtered and used for HPLC. Each water extract was treated with n-hexane, filtered and used for HPLC.

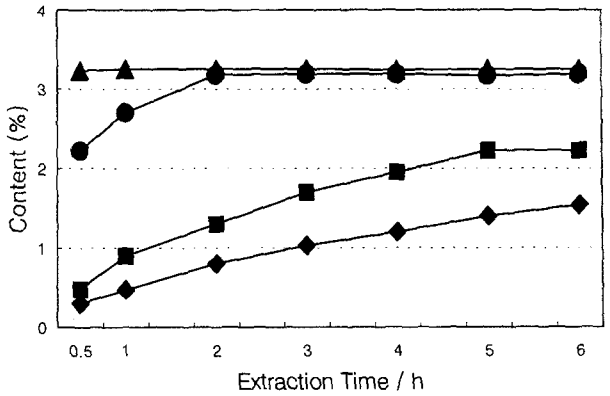

Fig. 3. Extraction yield of amygdalin according to cutting size of outer shell-eliminated tōnin with methanol.
▲ :crude powders, ●:small pieces, ■ :half pieces, □:whole pieces

Effects of emulsin on the extraction of amygdalin

To find out the major existing part of emulsin and the action mechanism, the content of amygdalin was determined by HPLC. Samples used were three kinds of tōnin as follows: natural formula, outer shell-eliminated and inner shell-eliminated tōnin. Each sample was prepared as the form of crude powder, refluxed with 50 mL of distilled water for 2 h, filtered and used for HPLC.

RESULTS AND DISCUSSION

Chromatographic behavior of amygdalin

Fig. 1 is a chromatogram of amygdalin from tōnin by reversed-phase separation with a 25% methanol as a mobile phase after extraction with water. The peak of amygdalin was completely separated in our method without any pretreatment.

The calibration curve between peak area and the concentration of standard amygdalin showed excellent linearity (r^2=0.9996).

Amygdalin extraction yield according to cutting size of tônin with methanol

Fig. 2 shows extraction yield of amygdalin by cutting

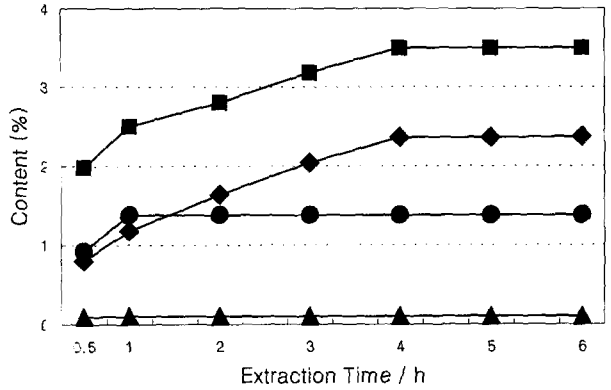

Fig. 4. Extraction yield of amygdalin according to cutting size of *natural formula tōnin with water.*
▲ :crude powders, ●:small pieces, ■ :half pieces, □:whole pieces

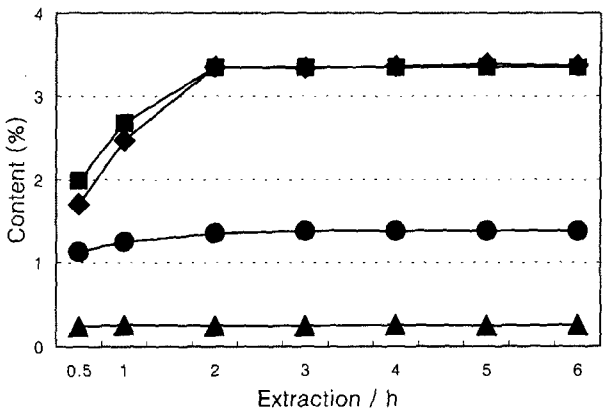

Fig. 5. Extraction yield of amygdalin according to cutting size of outer shell-eliminated tōnin with water.
▲ :crude powders, ●:small pieces, ■ :half pieces, □:whole pieces

sizes of natural formula tōnin with methanol. The extraction yield of amygdalin was 2.8% from crude powders, 2.8% from small pieces, 1.6% from half pieces and 0.5% from whole pieces. The extraction yields from crude powders and from small pieces were almost the same, but the time to take for extraction was 0.5 h from crude powders and 2h from small pieces. This result indicated that extraction yield and extraction rate were better from smaller size. However, extraction yields were generally low due to the husk.

Fig 3 shows extraction yield of amygdalin by cutting sizes of outer shell- eliminated tōnin with methanol. The extraction yield of amygdalin was 3.2% from crude powders, 3.2% from small pieces, 2.2% from half pieces and 1.5% from whole pieces. Extraction yield and extraction rate were better from the small size because emulsin, a hydrolyzing enzyme, almost didnt work in methanol, an organic solvent.

Compared with natural formula tōnin, the patten of extraction yield according to cutting sizes is similar, but the extraction yield itself was generally much higher.

Amygdalin extraction yield according to cutting size of tōnin with water

Fig. 4 shows extraction yield of amygdalin by cutting sizes of natural formula tōnin with boiling water. Different from the pattern of extraction yield by methanol, the extraction yield of amygdalin was 0.1% from crude powders, 1.4% from small pieces, 3.5% from half pieces and 2.4% from whole pieces. It was found that the amygdalin from crude powders of tōnin was mostly decomposed by emulsin and the effect of emulsin was decreased with the increase of the cutting size. In particular, the amygdalin from half pieces of tōnin was mostly extracted without interference of emulsin, but it had a shortcoming taken 4 h for complete extraction.

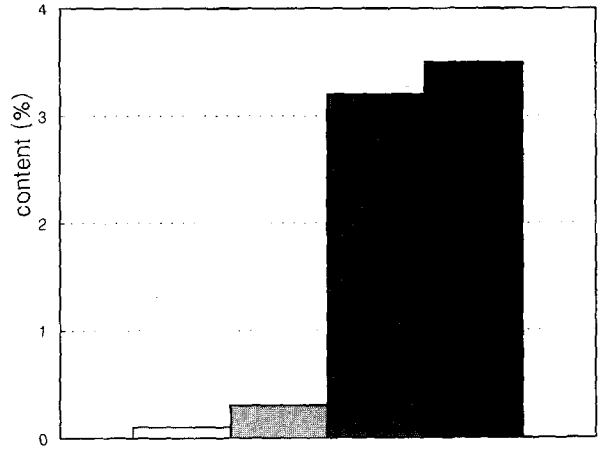

Fig. 6. Effects of emulsin on extraction of amygdalin (extraction time: 2h).
:powder form of natural formula tōnin
:powder form of outer shell-eliminated tōnin
:powder form of inner shell-eliminated tōnin
:half pieces or whole pieces of outer shell-eliminated tōnin

Fig. 5 shows extraction yield of amygdalin by cutting sizes of outer shell-eliminated tōnin with boiling water. The extraction yield of amygdalin was 0.3% from crude powders, 1.4% from small pieces, and 3.5% from half pieces and whole pieces respectively. The amygdalin from half pieces and whole pieces of tōnin was completely extracted at 2h without interference of emulsin. Therefore, we could find that emulsin had no effect for the decomposition of amygdalin in the use of sizes larger than half.

Effects of emulsin on the extraction of amygdalin

Fig. 6 shows the results of the study to find out the major existing part of emulsin and the action mechanism of emulsin in water. Extraction yield of amygdalin was

about 0.1% from the powder form of natural formula tōnin, 0.3% from powder form of outer shell-eliminated tōnin, and 3.2% from powder form of inner shell- eliminated tōnin, and 3.5% from half pieces or whole pieces of outer shell-eliminated tōnin.

Amygdalin was almost decomposed by emulsin in using crude powders of natural formula or outer shell-eliminated tōnin, because emulsin was adjacent to amygdalin and decomposition of amygdalin occurred faster than deactivation of emulsin by boling water.

Amygdalin was obtained more than 90% in using crude powder of inner shell-eliminated tōnin, because emulsin contained in inner shell part being removed previously before extraction.

Amygdalin was obtained up to almost 100% without interference of emulsin in using half pieces or whole pieces of outer shell-eliminated tōnin, because emulsin mainly contained in inner shell part was first extracted and deactivated by boiling water before extraction of amygdalin.

In this study, we could find out that emulsin was mainly contained in the inner shell part of tōnin and extraction yield of amygdalin was most high in the use of the size larger than half. Also, we could almost get amygdalin efficiently without pretreatment to remove emulsin by this method.

CONCLUSION

Tōnin has been used as crude powders in the prescription (water decoction) of traditional oriental medicine, but the method by crude powders of tōnin showed low extraction yield because of the decomposition of amygdalin by emulsin. However, our new method by half pieces or whole pieces of tōnin offered quantitative extraction yield because of no decomposition of amygdalin by emulsin.

It is thought that this method would be useful in the development of tōnin as anti-tumor agent.

ACKNOWLEDGEMENTS

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