J. Trad. Med. **24**, 193－199, 2007

Medical and
Pharmaceutical Society
for WAKAN-YAKU

# Differences in volatile compounds between tincture and Ayurvedic herbal liquor "Asava" made from ginger or jujube

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*(Received October 12, 2007. Accepted December 10, 2007.)*

**In Ayurvedic Medicine, some crude drugs and sugar are mixed and fermented, and developed into a herbal liquor called "Asava". During the alcoholic fermentation, the constituents of the crude drug would change chemically. There would thus be a chemical difference between Asava and tincture prepared by soaking the crude drug in alcohol. To investigate the differences between Asava and tincture, we attempted to prepare Asava in our laboratory with ginger rhizomes or jujube fruits and compared the volatile compounds of Asava with those of tincture by GC - MS. The alcohol contents of Ginger Asava and Jujube Asava prepared in the laboratory were 11 % and 9.0 %, respectively, and were almost the same as Asavas obtained in Sri Lanka. A characteristic difference between the Asava and the tincture was the relative content of phenylethanol (Asava: >30 %, tincture: <2 %), which is known to be a metabolite of phenylalanine during alcoholic fermentation. In the Ginger Asava, only trace aldehydes such as geranial and neral were found, whereas they were abundant in ginger tincture. Geranial and neral seemed to be reduced to geraniol and nerol. In Jujube Asava, we detected benzyl alcohol that was not detected in jujube tincture. Benzyl alcohol is an aglycon of zizybeoside I or II that was a component of jujube. Therefore, it seemed that glycosides are hydrolyzed by alcoholic fermentation. Hydrolysis of glycosides might help absorption of effective aglycones for the aged.**

**Key words** Ayurveda, Asava, herbal liquor, alcoholic fermentation.

## Introduction

Asava is a herbal liquor used in Ayurveda (Indian traditional medicine) and is prepared by a process of alcoholic fermentation of herbs. There are a number of types of Asava according to the prescribed ingredients such as Candanaasava, Kumaryasava, Lohasava, *etc.*,[1)] and they are used mainly for patients with indigestion or emaciation.

Herbal liquors are also consumed in Japan, especially by the aged as tonic. However, Japanese herbal liquors are only prepared by soaking the crude drug in alcohol, making a tincture.

A number of investigators have studied the effects of fermented foods[2-4)] like Natto, wines, and yogurts, *etc.* Those fermented foods have different chemical compositions and, thus, different nutrition values. Similarly, Asava would be pharmaceutically different from tincture, which contains an intact set of herbal ingredients. However, very few studies have been reported on Asava and the other fermented herbal liquors[5,6,9)] and no comparison of Asava and tincture has been made to date. The objective of this study was to investigate the differences between Asava and tincture. At first, we researched the method of preparation of Asava in Sri Lanka. It was carried out from 20th to 22nd February 2006 at the Bandaranyke Memorial Ayurvedic Institute (BMARI), Nawinna, Maharagama. Subsequent to the field research, we attempted to prepare Asava in our laboratory following the general procedure in BMARI.

Recent studies on wines have shown that *Saccharomyces cerevisiae,* known as "brewer's yeast", hydrolyzes glycosides and releases aglycones such as monoterpenols and sesquiterpens which impact the wine aroma.[8)] It was thus expected that the profiles of volatile compounds would also change by fermentation in Asava. In this paper, we report the differences in the volatile compound profiles between Asava and tincture of Ginger or Jujube. These crude drugs were chosen because they are mentioned as ingredients of Asava in the Sushruta Samhita,[9)] bible of Ayurveda, and are seen in Japanese pharmacopoeia fifteenth edition (JP 15) also.

In Ayurvedic Medicine, there is another herbal liquor called "Arishta", prepared from fermentation of herb decoction. It would be difficult to compare Arishta with tincture because of heating of crude drugs. Since we compared Asava prepared from fermentation of herb with the tincture in present study.

## Materials and Methods

**Crude drug materials.** The following crude drugs were used in this study.

Small pieces (3 - 10 mm) of Ginger rhizomes (*Zingiber officinale* Roscoe [Zingiberaceae], lot No.: US262730) and Jujube fruits (*Zizyphus jujuba* Miller var. *inermis* Rehder [Rhamnaceae], lot No.: US312324) were purchased from UCHIDA WAKANYAKU CO., LTD. (Tokyo, Japan). These crude drugs are compliant to JP 15. Dried flowers of

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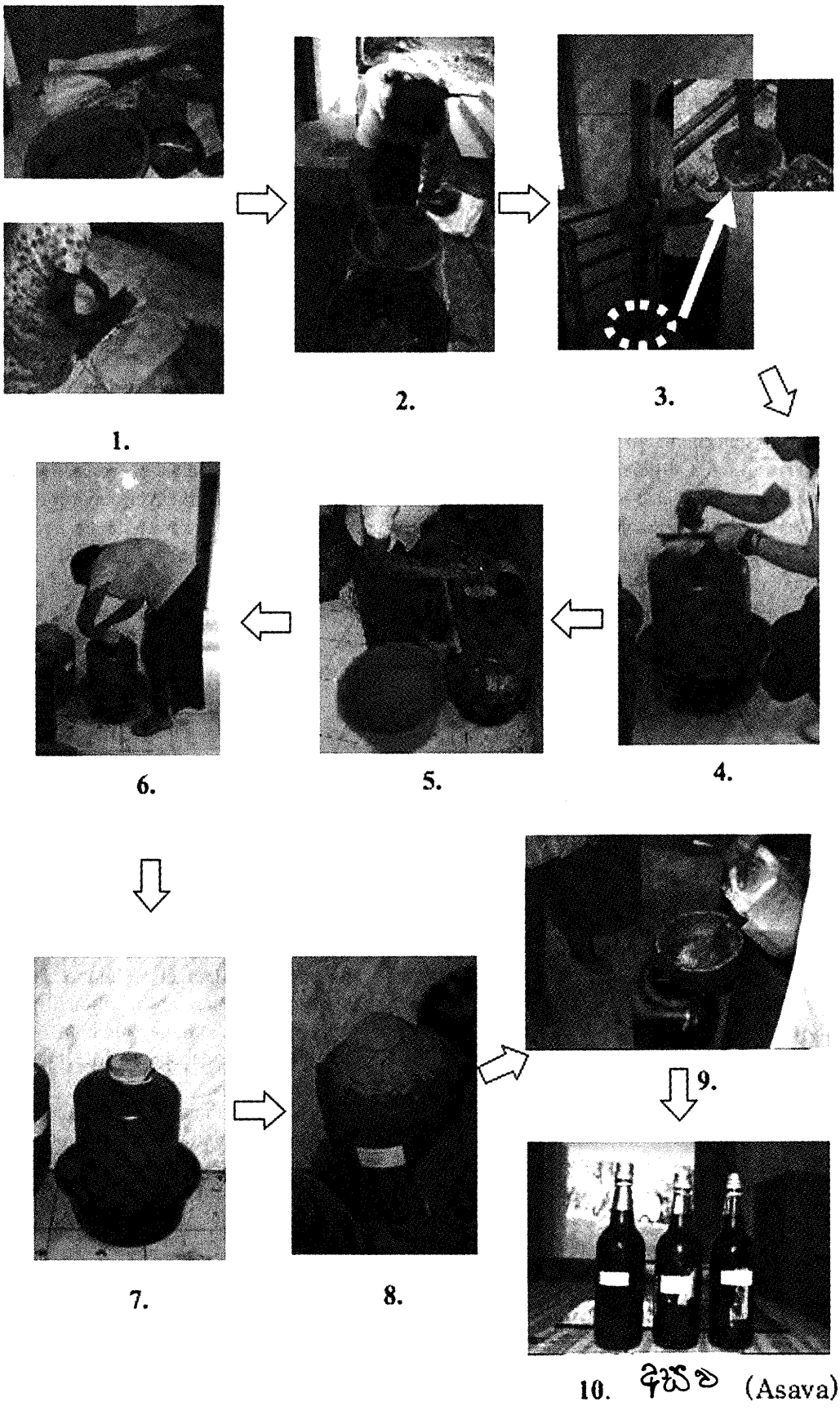



**Fig. 1** Preparation of Asava in Sri Lanka. **1.** 120 g of raw materials (20 - 30 crude drugs) of Asava were chopped with a knife. **2.** Chopped crude drugs were washed with water. **3.** They were ground into coarse powder with a mortar and a pestle. **4.** 120 g of maritta - maru (flowers of *Woodfordia floribunda*) and mixture of raw materials were put into an earthenware pot. **5.** 9 kg of white sugar was dissolved to 15 l of water and the solution was poured into the pot. **6.** The mouth of the pot was covered with cloth. **7.** A wooden lid was put on the mouth. They were stirred everyday during the first 1 week. **8.** After 1 week, the mouth was sealed with clay and the solution was brewed for about 1 month. **9.** The solution was filtrated with a funnel and a mesh. **10.** Three kinds of bottled Asava.

fire flame bush (*Woodfordia floribunda* Salib. (= *W. fruticosa* Kurz.) [Lythraceae], Voucher No. KANP06756) was purchased from KYAW KYAW Myanmar Trad. Med. Shop (Mandalay, Myanmar).

**Chemicals.** Phenylethanol, benzyl alcohol, sucrose, ethanol, pentane, and acetone were purchased from Nakalai Tesuque (Kyoto, Japan). Nerol and geraniol were purchased from Millennium Chemicals (USA). Geranial and neral were purchased from Kuraray (Tokyo, Japan).

**Preparation of the Asava.** Following the procedure in BMARI (Fig. 1), we determined the protocol of making Asava as follows.

1. We mixed 20 g of ginger or jujube, 68 g of sucrose and 2 g of flowers of *W. floribunda* with 115 ml of distilled water. The amounts of crude drugs and sucrose concentration were determined based on field research in Sri Lanka.
2. These liquids were maintained at 25℃ for 1 week or 5 weeks in an incubator (LPH 200 - RDSMP, Nippon Medical & Chemical Instruments CO., LTD. Japan).

**Preparation of the tincture.** 20 g of ginger or jujube were added to 115 ml of 14.0 % (v/v) ethanol aq. and these liquids were maintained at 25℃ for 35 days in the incubator.

**Examination of sugar contents and pH.** We measured sugar contents by Pocket PAL - 1 (Brix : 0.0 - 43.0 %, ability of decomposition : Brix 0.1 %, precision of measurement : Brix 0.2 %, range of temperature revision : 10℃ - 60 ℃) and pH by HORIBA twin pH B211 on days 0, 1, 2, 3, 4, 5, 6, 7, 14, 28 and 35.

**Examination of alcohol contents.** We measured alcohol contents according to the method prescribed by the National Tax Agency of Japan.[10)]

(1) An aliquot of 100 ml of the Asava or tincture was directly distilled until 70 ml of distilled liquid was acquired.
(2) Distilled water was added to the distilled liquid and adjusted to 100 ml.
(3) The distilled liquid was poured into a glass cylinder and an alcohol meter (range: 0 - 30, minimum scale: 1, Yokota Seisakusho, Japan) was floated on this distilled liquid.

**Characterization of the volatile compounds.**

1. Extraction Procedure

An aliquot of 100 ml of the Asava or tincture was directly distilled for 40 min and 20 ml of distilled liquid was extracted with a mixture of pentane: acetone = 9 : 1. The organic layer was evaporated to yield a residue with an evaporator.

2. GC - MS Analysis

The GC - MS unit consisted of an Agilent 6890 gas chromatograph coupled with an HP 5973 mass selective detector (Agilent USA). A fused silica capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness) coated with Agilent technology HP5MS bonded methylpolysiloxane was used. The chromatographic conditions were as follows: initial temperature,40℃ ramped at a rate of 5℃/min to 100 ℃, at 8℃/min to 150℃ and at 10℃/min to 250℃ using helium as the carrier gas (column head pressure = 18 psi, flow rate = 1 ml/min). The temperature of the injector and transfer line was held at 250℃. Compounds detected were identified by comparing their mass spectra with those in the database (NIST libraries) and retention times made with those of reference standards (benzyl alcohol, phenylethanol, nerol, neral, geraniol and geranial). To improve the sensitivity of MSD, SIM mode was applied subsequent to SCAN mode. Selected ions were *m/z* 103 and 154 for 1,8 - cineole, *m/z* 108 and 79 for benzyl alcohol, *m/z* 122 and 91 for phenylethanol, *m/z* 154 and 110 for borneol, *m/z* 154, 69 and 93 for nerol and geraniol, *m/z* 69 and 152 for neral and geranial, *m/z* 93, 119, and 204 for curcumene and zingiberene.

## Results and Discussions

**Asava preparation in laboratory.** Time courses of changes of the sugar content and the pH of Asavas during 35 days of fermentation are shown in Fig. 2. The pH of Asava began to decrease after 2 days. The sugar content began to decrease after 3 or 5 days and the alcohol contents

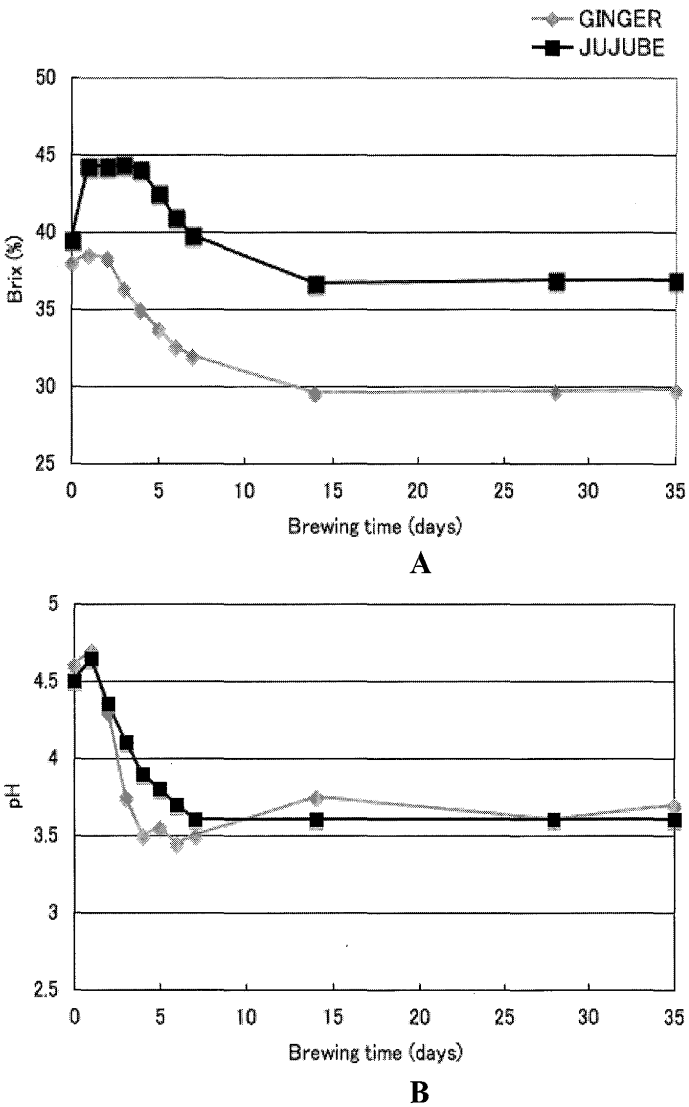



**Fig. 2** Time course of change of the sugar contents **A** and the pH **B** of Asava prepared in our laboratory.

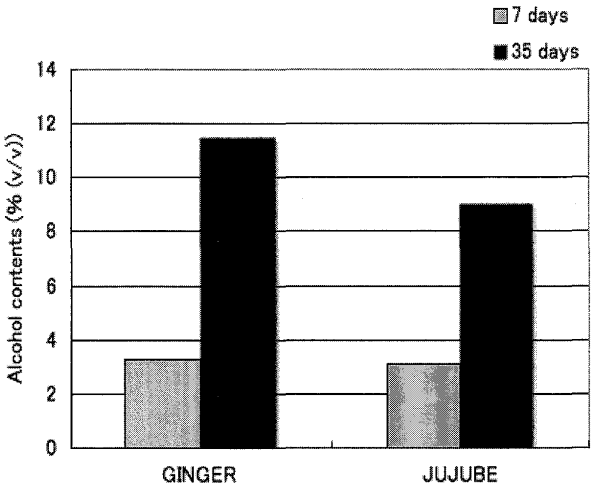
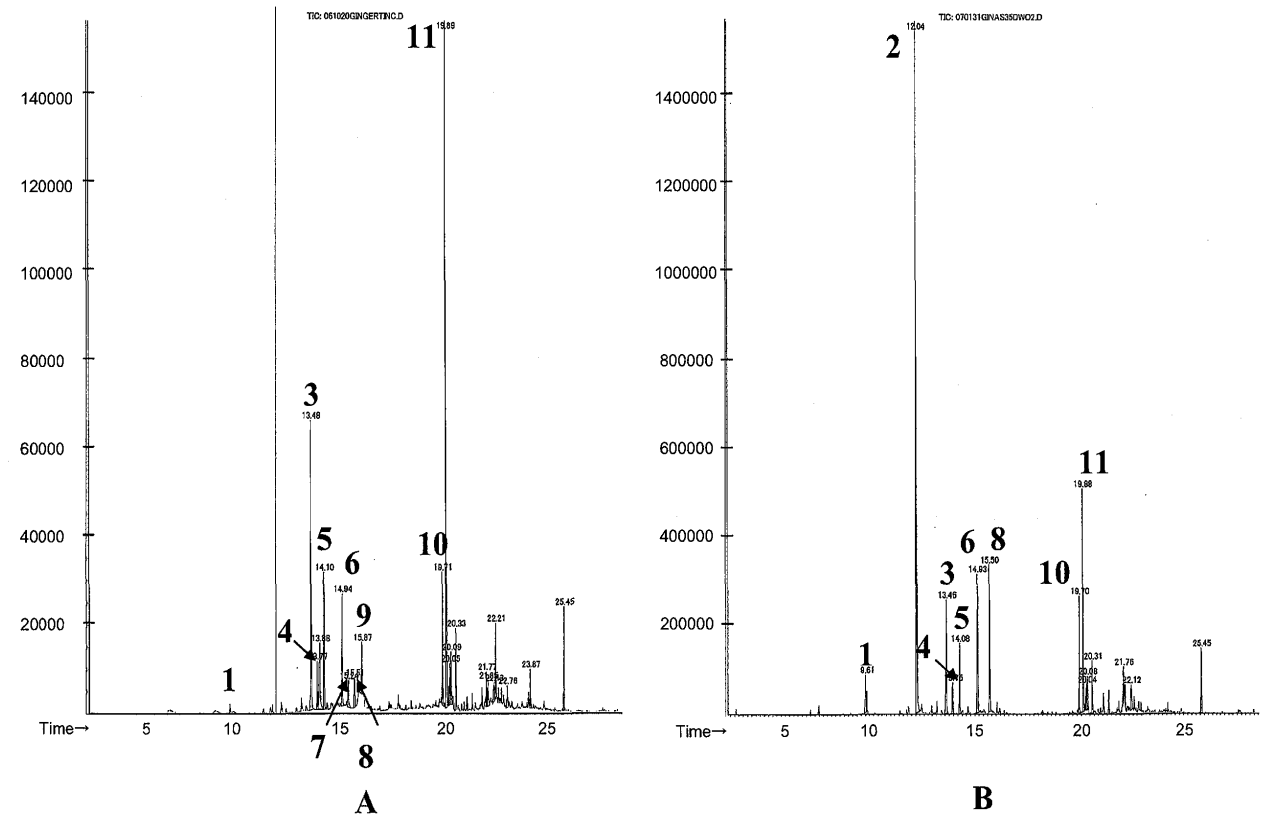



**Fig. 3** Alcohol contents of Asava in our laboratory.

**Table 1** The alcohol contents, sugar contents and pH of Asava prepared in our laboratory and BMARI.

| | Alcohol content (%(v/v)) | Sugar content Brix(%) | pH |
|---|---|---|---|
| Ginger Asava | 11.5 | 29.8 | 3.7 |
| Jujube Asava | 9.0 | 36.9 | 3.6 |
| Pippalyadyasava | 7.9 | 29.6 | 3.6 |
| Aravindasava | 9.1 | 27.2 | 3.7 |
| Punarnawasava | 9.1 | 26.5 | 3.7 |



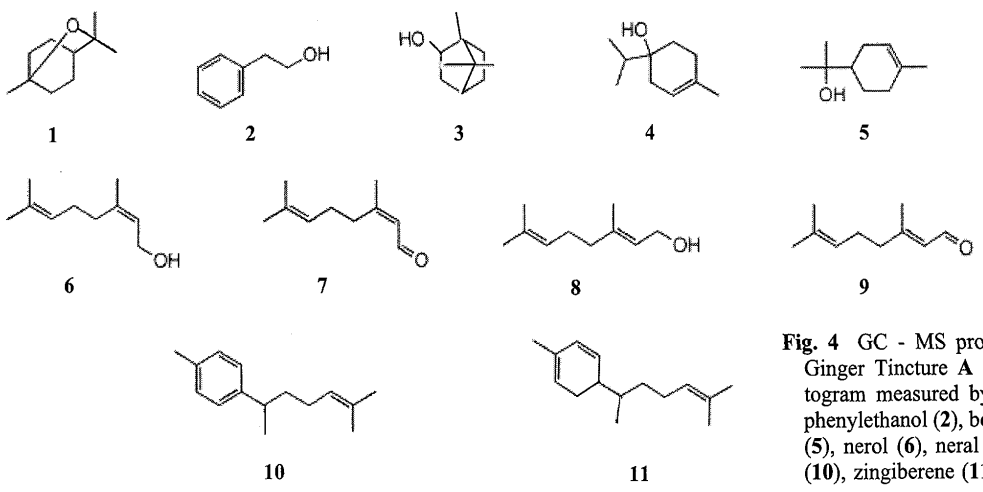




**Fig. 4** GC - MS profiles of volatile compounds contained in Ginger Tincture **A** and Ginger Asava **B**. GC - MS chromatogram measured by a SIM mode. Peaks: 1,8 - cineole (**1**), phenylethanol (**2**), borneol (**3**), terpinen - 4 - ol (**4**), α- terpineol (**5**), nerol (**6**), neral (**7**), geraniol (**8**), geranial (**9**), curcumene (**10**), zingiberene (**11**)

were 11 % (Ginger) and 9.0 % (Jujube) after 35 days (Fig. 3). The sugar and alcohol contents, and pH of Asava obtained in Sri Lanka are shown in Table 1. These values were almost the same as for the Asava prepared in this study.

**Analysis of volatile compounds in Asava and tincture prepared with Ginger.** Phenylethanol, which accounted for about 30 - 90 % of volatile compounds of all Asava (Fig. 4, 6), was also contained in Asavas obtained in Sri Lanka (Pippalyadyasava: 26.0%, Aravindasava: 42.2%, Punarnawasava: 52.6%). In addition, the relative content increased from 1 to 5 weeks in Ginger Asava (Fig. 5). It is known that phenylalanine is convertible to phenylethanol by yeasts during fermentation.[11] Thus, the flavor of Asavas prepared in our laboratory was similar to that of Asavas prepared in Sri Lanka. Since the relative content of phenylethanol in tinctures was less than 2.00%, the presence of phenylethanol is characteristic of Asavas.

In Ginger Asava, only traces of aldehydes such as geranial and neral, which were contained in Ginger tincture (Table 2), were found. In contrast, the relative contents of geraniol and nerol in the Asava were higher than in tincture. There was a report on reduction of ketone to alcohol by baker's yeast.[12] Thus, it was thought that yeasts reduced geranial or neral to geraniol or nerol. Another possibility is that the yeast hydrolyzed the geraniol glycosides. It has been reported that disaccharides of geraniol are present in ginger.[13] Thus, geraniol would be released during fermentation by hydrolysis of geraniol disaccharides. Although some aldehydes have pharmacological effects, these compounds are also toxic. It has been reported that geranial and neral, which are added to foods as a flavoring agent, cause forestomach and kidney lesions.[14] Asavas, which contain monoterpenols in place of such aldehydes, could be a milder drug than tincture.

**Table 2** Area percent of the volatile compounds contained in Ginger Tincture and Ginger Asava. "r.t." means retention time.

| | | r.t. (min) | Area % | |
|---|---|---|---|---|
| | | | Tincture | Asava |
| 1,8-cineole | **(1)** | 9.690 | 0.34 | 0.98 |
| phenyl ethanol | **(2)** | 12.05 | 0.53 | 34.0 |
| borneol | **(3)** | 13.47 | 12.6 | 5.29 |
| terpinen-4-ol | **(4)** | 13.76 | 1.99 | 1.35 |
| α-terpineol | **(5)** | 14.09 | 5.26 | 2.82 |
| nerol | **(6)** | 14.94 | 4.65 | 5.94 |
| neral | **(7)** | 15.24 | 1.53 | 0.26 |
| geraniol | **(8)** | 15.50 | 1.51 | 6.05 |
| geranial | **(9)** | 15.87 | 4.37 | 0.50 |
| curcumene | **(10)** | 19.71 | 4.74 | 4.02 |
| zingiberene | **(11)** | 19.89 | 20.5 | 7.20 |

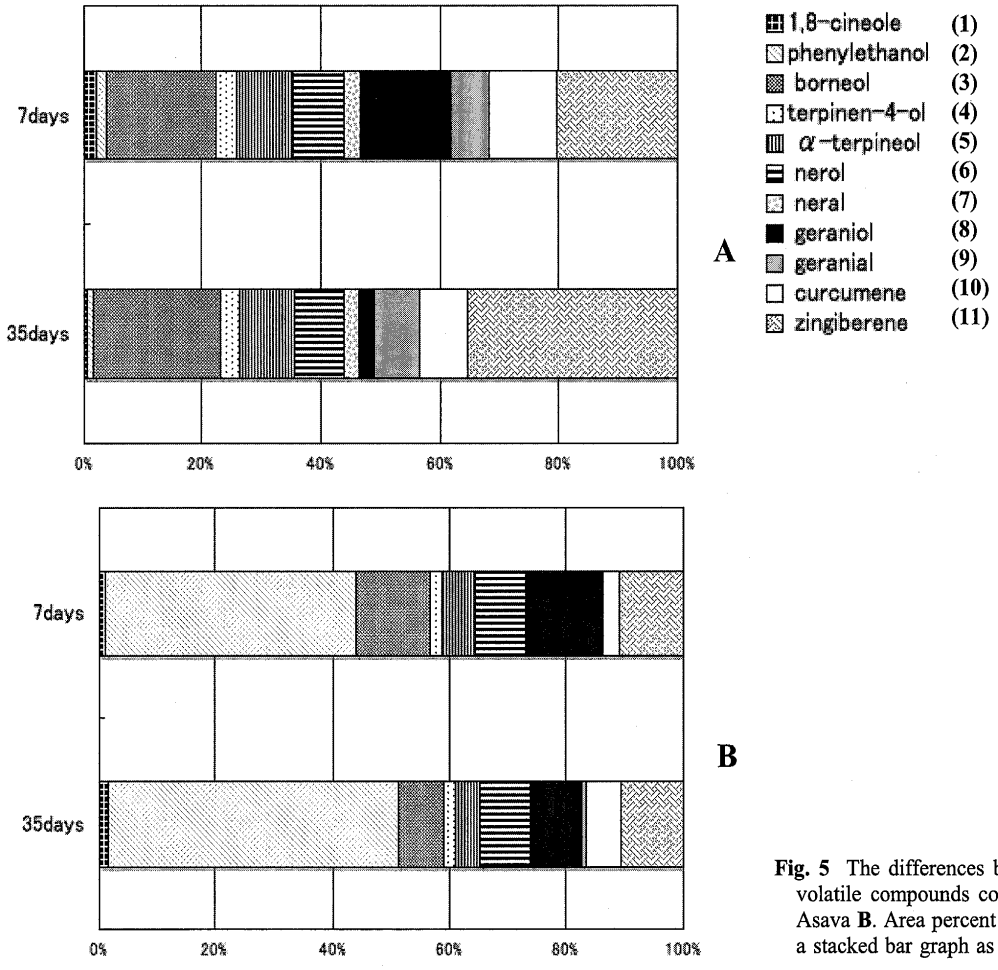



**Fig. 5** The differences between 1 week and 5 weeks of major volatile compounds contained Ginger Tincture **A** and Ginger Asava **B**. Area percent of major 11 compounds was applied to a stacked bar graph as a percent of the total.

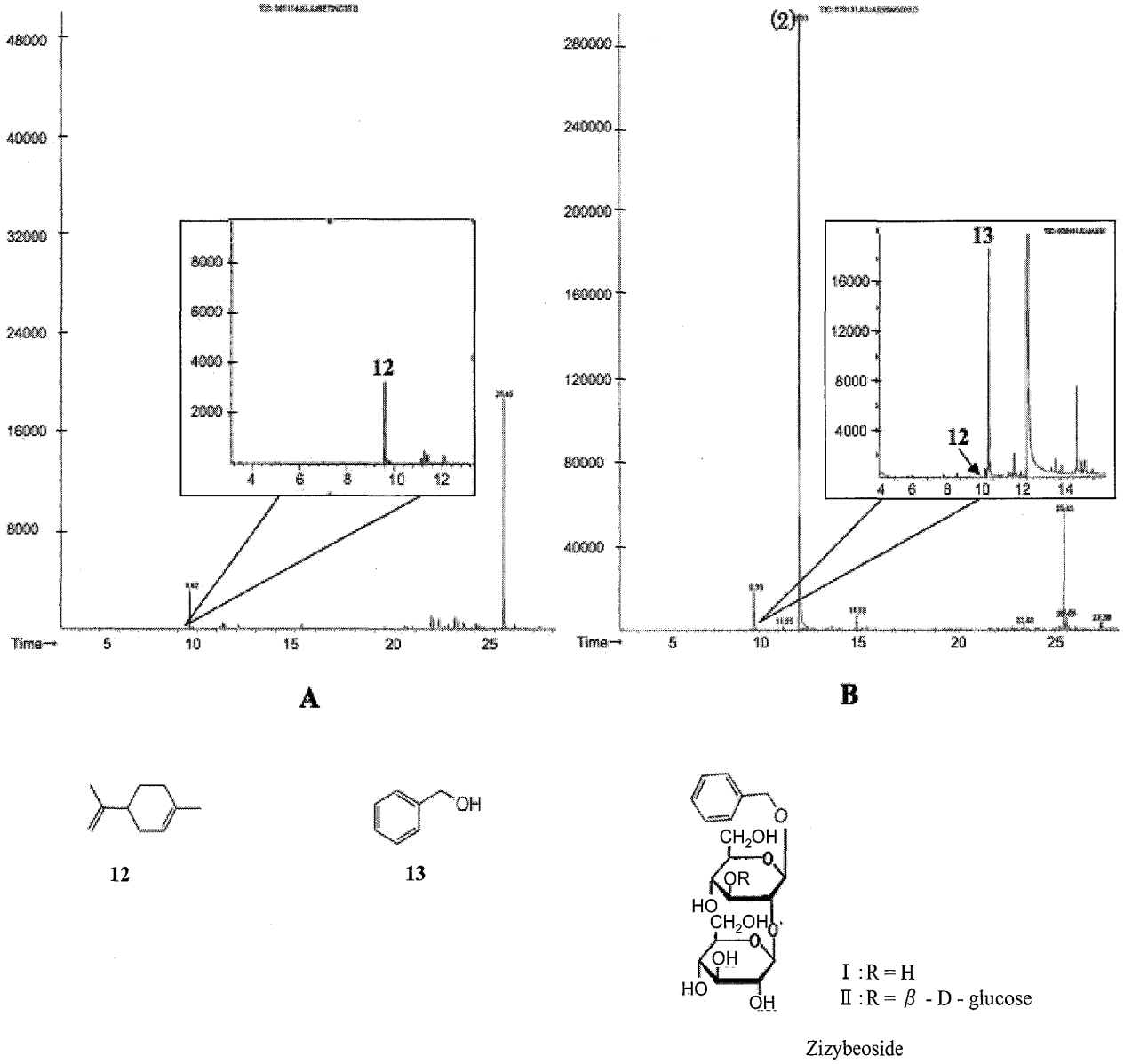



**Fig. 6** GC - MS profile of volatile compounds contained in Jujube Tincture **A** Jujube Asava **B**. GC - MS chromatogram measured by a SIM mode. Peaks: *d* - limonene (**12**), benzyl alcohol (**13**), phenylethanol (**2**)

**Analysis of volatile compounds in Asava and tincture prepared with Jujube.** The tincture contained few volatile compounds, as the levels of all volatile compounds were less than 4000 (Fig. 6). However, while benzyl alcohol was present in Asavas, it couldn't be detected in the tincture. From 7 to 35 days of fermentation, the relative content of benzyl alcohol slightly increased (0.12 → 3.32%).

Benzyl alcohol is an aglycon of zizybeoside I or II, a characteristic component of Jujube, and was presumed to be a hydrolyzed product of zizybeoside in Jujube Asava.

Herbs contain a number of glycosides but the intestine hardly absorbs glycosides. Aglycons can be absorbed and have pharmacological effects. Glycosides can be effective after hydrolysis by β- glucosidase and β- glucuronidase of human intestinal bacteria such as *Eubacterium* sp. and *Bifidobacterium* sp..[15] However, aged people are reported to have less of these intestinal bacteria, and therefore they tend to suffer from indigestion. Hydrolysis of glycosides by fermentation in Asava, suggested in the present study, might promote the absorption of effective aglycones in the aged.

## Conclusion

We showed that the profile of volatile compounds of Asava were different from that of tincture. Especially the relative content of geraniol in Ginger Asava and benzyl alcohol in Jujube Asava were higher than that of tincture. Therefore, it was suggested that glycosides were hydrolyzed by fermentation in Asavas. Hydrolysis of glycoside would be characteristic reaction in Asava and the reaction might promote the absorption of glycosides contained in crude drugs by the intestine.

## Acknowledgements

We are grateful to Dr. R. Thiranagama and Dr. S. Perera, Bandaranayke Memorial Ayurvedic Institute, for their help during the fieldwork in Sri Lanka. Sincere thanks are offered to Ms. T. Tamura, Kaihatsu Management Consulting, Inc. for her heartful help in making the appointment with BMARI. We wish to thank Mr. N. Hayashi, Libra Natutherapy CO., LTD for allowing us to use GC - MS.

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## Japanese abstract

Ayurvedaで古くから薬用に供される「Asava」は，生薬を発酵させて製する薬酒である。我が国における一般的な薬酒は生薬を酒に浸漬させてつくられ，これら両者の大きな違いは発酵という過程を経ているか否かという点である。発酵が薬酒の性質にどのように影響を与えるかを明らかにするため，本研究では生姜と大棗を用い，それぞれからAsava製法とチンキ剤製法により薬酒を作製し，揮発性成分比をGC-MSにより測定した。Asava製法については，スリランカにて調査した結果に従った。その結果，Asava製法で作製した薬酒の揮発性成分比は，phenylethanolが30％を占めたのに対し，チンキ剤製法では2％以下と大きく異なっていた。またAsava製法で作製した生姜の薬酒はアルデヒド類(geranial, neral)が1％以下であり，作製中にgeraniol, nerolに還元されたことが示唆された。さらにAsava製法で作製した大棗の薬酒には，zizybeosideのアグリコンであるbenzyl alcoholの含有が認められたことから，アルコール発酵による配糖体の加水分解が示唆された。

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