CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The objectives of this research were to investigate the effects of HPP and thermal treatment of pennywort juice on the bioactive compounds, flavour components, physicochemical properties and microbiological quality during storage, as well as the effect of sugar addition on these parameters. Investigation of the suitable juice processing conditions by HPP involved finding the optimal values of high pressure level and holding time. For thermal processing, including pasteurization and sterilization, the aim was to find the optimal temperature and heating time. The conclusions obtained from this research are as follows:

1. The most suitable processing condition based on microbiological quality of HPP was 400 MPa for 20 min at room temperature (<30˚C). Also, suitable thermal processing conditions were identified for pasteurization by heating at 90˚C for 3 min and for sterilization by heating at 121˚C for 4 min. The content of microorganisms in all processed juice was less than the limits recommended in Thai Food Regulation-Standard (2003). Analysis of the fresh juice indicated only total plate count, yeasts and moulds, which may be limited due to some biological properties, such as the effect of asiaticoside on antibacterial and fungicidal activity.

2. There were losses of bioactive compounds, including madecassoside, asiaticoside, chlorophylls, ascorbic acid, and total phenol content, and a reduction in FRAP values due to the processing of pennywort juice. HPP juice had the smallest losses of ascorbic acid and antioxidant capacity but further changes on storage indicated that the process had not inactivated all microorganisms.

3. Colour degradation occurred after processing due to the loss of chlorophyll and non-enzymatic browning (Maillard) reactions during thermal treatment. The enzymatic browning reaction in HPP treated samples indicates that the HPP had not completely inactivated PPO.

4. Sucrose addition caused an increase in the total soluble solids of the juice. After juice processing by HPP and thermal treatments, the viscosity, the content of madecassoside, ascorbic acid, chlorophyll, *β*-carotene and the antioxidant capacity (in terms of total phenolic content and FRAP) of this juice with added sugar was higher than that for the juice without sugar. In contrast, the pH value and the colour, in terms of chroma, of juice with added sugar were lower than those of the juice with no sugar.

5. There were losses of ascorbic acid, total phenol content and FRAP values of all processed juice during storage at 4˚C for HPP and pasteurized juices, and for sterilized juice stored at 40˚C. However, loss of *β*-carotene was only found in sterilized juices.

6. The effects of processing on the microbiological properties of processed juices during storage for 4 months showed satisfactory results. *S. aureus* and *C. perfingens* were not detected and *E. coli* was less than 2.2 per 100 ml and also yeasts and moulds count was less than 30 cfu/ml in all samples, except for the total plate count of pressurized samples. The total plate count of HPP treated juice without sugar addition increased from 35 to 187 cfu/ml during 9-16 weeks of storage. Also, the HPP treated juice with sugar addition had total plate count increased from 31 to 258 cfu/ml during the 7-16 weeks of storage. Addition of sucrose may contribute to the multiplication of bacteria by acting as a carbon source.

7. The flavour compounds in fresh pennywort juice contained 48 volatiles. High pressure treated juice without sugar contained 49 volatiles, whereas with sugar addition the juice contained 56 volatile compounds. Pasteurized juice and sterilized juice without sugar contained 55 volatiles, whereas juice with sugar addition contained 60 and 74 volatiles in pasteurized and sterilized juices, respectively. This means that sugar brings about an increase in the number of volatile components through the interaction with other compounds in the food matrix. In addition, juice with sugar addition contained pentanal and heptanal after HPP and sterilization, respectively.

8. The total concentration of volatile compounds in fresh juice was higher than in processed juice without added sugar ($p \leq 0.05$) and there was a non-significant change in processed juice with added sugar. The fresh juice was characterized by a high content of the acyclic alcohols, aldehydes, oxygenated monoterpenes and

oxygenated sesquiterpenes, which were present at higher concentrations in the fresh juice than in other processed juices.

9. Some volatile components were retained better by HPP than by pasteurization or sterilization. HPP caused more flavour volatiles in the acyclic alcohol class to be retained, with a trend to increased retention of aldehydes and oxygenated monoterpenoids compared to pasteurization and sterilization. Many compounds were conserved better by HPP treatment including linalool, geraniol, *α*-copaene, *β*-selinene and cuparene. Some compounds, including 2,6-nonadienal, 2-nonenal, *β*-cyclocitral, *γ*-cadinene and caryophyllene oxide were present in fresh juice but were lost during HPP. Some of the compounds, including 2-butanone, 3-nonen-2-one, *γ*-terpinene, *α*-terpineol and tetrahydrofuran were not present in fresh juice but were found in HPP juice, and this indicates that HPP can induce chemical changes which generate new compounds from components of the fresh juice. Several compounds, including 2-nonanone and *α*-ylangene were detected in heat-treated juice but were not found in fresh and HPP juice. Although the total volatile concentration in sterilized juice was higher than in fresh and processed juices ($p>0.05$) by HPP and pasteurization, some volatile components that were not present in the fresh juice were formed at high levels in the sterilized juice such as *γ*-terpinene, ketones, and germacrene *D*.

10. Acyclic alcohols were found in high concentration in fresh and HPP juice. Hexanol, which can be formed by alcohol oxidoreductase activity on hexanal, was only detected in HPP. These compounds might be formed during juice preparation because no action was taken to inactivate enzymes prior to extraction and/or enzyme activation can arise from pressure-induced effects.

11. Some aldehydes, which are other lipid oxidation products including 2-nonenal and 2,6-nonadienal, have been used to indicate oxidized flavor, and these were found in fresh juice but were not found in processed juices. Some aldehydes, including 2-methyl-propanal, 3-methyl-butanal, 2-methyl-butanal, 2,6-nonadienal and 2-nonenal, decreased or disappeared after thermal treatments, whereas the known lipid oxidation product, hexanal, was found in sterilized samples in higher concentrations than in pasteurized and HPP juices. Losses of aldehydes may be due to

degradation by chemical reactions including acetal formation which occurs in the presence of alcohols under acid conditions.

12. Ketones were present in processed juice but were not found in the fresh juice.

13. Some monoterpene hydrocarbons such as terpinolene and *α*-terpinene were found in heat-treated samples. These studies showed that *γ*-terpinene, which contributes bitter flavours, was found in higher concentration in processed samples. *β*-Pinene was found at higher concentration in fresh pennywort juice than in processed samples.

14. Some oxygenated monoterpenes like *α*-terpineol are perceived as off-flavours, and *α*-terpineol, which is known to be formed from limonene and linalool by acid-catalyzed reactions, was found in high concentration in HPP samples. Some oxygenated monoterpenes like linalool were found to be reduced by the thermal processing of juice.

15. The sesquiterpene class, including *β*-caryophyllene, humulene, *E-β*-farnesene, *α*-copaene, alloaromadendrene and *β*-elemene, was the major class of volatiles present in this juice. Some of the compounds were detected in the processed juice including calamenene have not been reported previously in pennywort juice.

16. Some oxygenated sesquiterpenes like caryophyllene oxide were only detected in the fresh juice.

5.2 Recommendations for further investigation

1. A further study to extend the shelf life of the processed juice by adding some preservatives such as ascorbic acid to reduce the pH of the products is recommended. A study to apply preservatives would be another alternative to prevent microbial growth during the storage.

2. The method of juice extraction that involved a long time with air and light-exposures should be improved for optimal colour preservation.

3. A further investigation of the quality and quantity of the by-products should be justified to obtain evaluation of the economics of pennywort juice manufacturing