Application of Superheated Steam for Utilization of Japanese Cedar Resources

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論 文 内 容 要 旨

Cedar is the largest tree species in Japan, accounts for 18% (4520 kha) artificial forest in the country. Although cedar forest has contributed to maintaining the relevant ecological (including biological diversity), economic, and social function, the large-scale cedar forest has brought some worries based on environment for Japanese, for example, the nationwide cedar pollen allergen and forest devastation and soil erosion caused by a lot of landslides in cedar forests when they meet heavy rainstorm. A lot of thined wood and discard wood have not been in use. To relieve the environmental risks and to enhance economic profit of cedar forests, other effective utilizations of cedar resources besides as the resources for energy or wood industry are desired. The volatile organic chemicals (essential oil) in the cedar have been reported to have various bioactivities such as antifungal, antimite, antitermitic and antimosquito, on the other hand, these chemicals induce P450-dependent microsomal enzyme in laboratory animals, and thus limit the application of cedar woodchips as beddings for laboratory animals.

As shown in Figure 1, superheated steam (SHS) is a high-enthalpy, low-density water vapor phase, and provides advantages including increased heat transfer, an oxygen-free circumstance, accelerated drying rate and improved energy efficiency. Therefore, SHS under the mild conditions (below 200 °C) is inferred to be effective medium for enhancing the extraction of organic substances from biomass. By now, only a few works have been

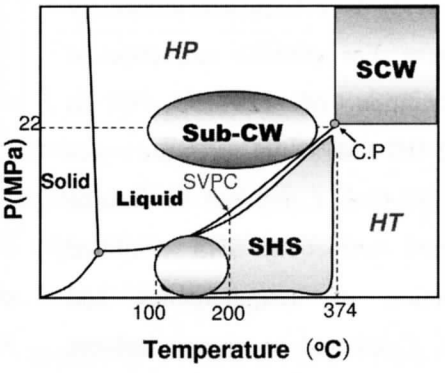


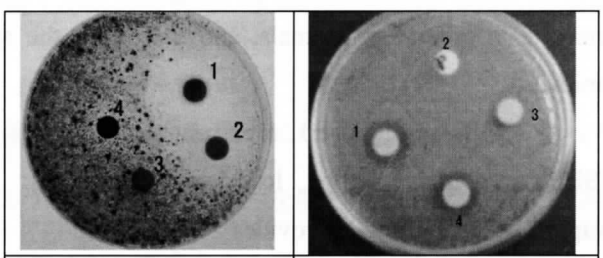
Figure 1 Water phase diagram

done for the use of SHS as an extraction medium. In this thesis, SHS has been developed as a novel extraction technique to utilize cedar resources. One is to extract the useful organic substances from fresh cedar leaves, and another is to convert cedar woodchips into a safe bedding material for laboratory animals by removing those volatile harmful organic compounds from woodchips. Therefore, this thesis consists of the following parts.

In chapter I, the research backgrounds were described. The aim and research method applied in this study were presented.

In chapter II, the extraction properties of SHS were investigated comprehensively. Firstly, a novel continuous extraction apparatus was developed. Then, extraction properties of SHS were investigated by determining the oil yield from cedar leaves under various temperature-pressure conditions. Extraction mechanism was investigated in detail based on the analysis of the heat transfer properties of SHS and the effects of water content in materials. As a result, SHS isolated a higher oil yield than that obtained by conventional steam distillation. Increasing temperature and pressure were found to improve the oil yield effectively. Analysis of extraction mechanism has revealed two points: i) because of its high heat transfer efficiency, SHS can transfer the more energy to a low-temperature material by condensation of water at the surface of materials; ii) because of the large heat capacity, water content contained in materials would absorb heat from extraction medium, and transfer more energy to those organic molecules around it leading to assistance of the extraction of those organic substances. Driving force derived from evaporation of intrinsic water and diffusion of oil itself promote the extraction of the essential oil in SHS.

In chapter III, because sesquiterpenes were thought to be the dominant compounds responsible for various bioactivities of essential oil of cedar, in this chapter, we developed the utilization of SHS on the selective extraction of sesquiterpenes from cedar leaves. In addition, to explore the utilization of the extracts obtained by SHS, their antimicrobial activities were examined by disk-diffusion method. The properties of SHS on selective extraction of organic substances were compared with those with hexane and methanol. As a result, the followings have been demonstrated: increasing temperature and pressure of steam are favorable to improve the yields of sesquiterpenes. A temperature more than 150 °C enhanced the extraction of diterpenes significantly. The extractions of some larger molecular-weight compounds (diterpenes and alkanes) and polar organic compounds (ester and carboxylic acid) have been accelerated by increasing steam pressure. For selective extraction of sesquiterpenes, a condition of 120~150 °C in temperature and 0.2~0.3 MPa in pressure was demonstrated to be effective. Compared to hexane extraction and methanol extraction, SHS exhibited selectivity on extraction of sesquiterpenes and sesquiterpene alcohols. Compared to organic solvent extracts, SHS extracts showed high antimicrobial



Alternaria sp.	*Escherichia coli*
1,2- SHSO[1-2]	1,4- SHSO[1-2]
3- HEO	2- HEO
4- MEO	3- MEO

Figure 2 Determination of growth-inhibiting haro of two microorganisms by cedar extracts (SHSO[1-2]: SHS extracts obtained at 170 °C and 120 °C; HEO: hexane extracts; MEO: methanol extracts.

activity. SHS is proved to be a promising method for selective extraction of some special active compounds against the growth of fungi, bacteria and mycetes.

In chapter IV, we developed the application of SHS as an extraction technique to convert cedar shaving into a kind of safe bedding for laboratory animals. In details, the optimum treatment condition was investigated, for not only effective removal of those harmful compounds for laboratory animals (volatile sesquiterpenes and aromatic hydrocarbons) from cedar bedding, also protecting and improving the properties of woody structure. And then, a large-scale practical apparatus was developed. As a result, the optimal conditions for the improvement of cedar bedding were 140 °C, 0.35 MPa and for 60 min. Under this condition, the total essential oil in cedar bedding can be effectively extracted by over 50%, and those volatile compounds such as sesquiterpene and naphathalene-derived aromatics can be almost removed. Also, under this condition SHS treatment increased the adsorption capacity of the cedar bedding for ammonia gas. Therefore, the improved cedar bedding is expected to improve the housing environment by lowering the ammonia concentration. These results indicated that the improvement of cedar bedding provides the possibility of the use of cedar beddings as laboratory animal bedding.

In chapter V, because cytochrome P450s enzymes (P450) play an important role in detoxification of xenochemicals including therapeutic drugs, countless toxins and carcinogens, in this chapter, we examined the effects of single oral administration of essential oil of Japanese cedar bedding (EOJC), as well as the effects of the improved cedar beddings from which partial EOJC had been removed, on the P450 enzyme systems in mice. In addition, the effects of the improved cedar bedding on the growth and reproductive properties of animals were examined through a long-term (17 weeks) housing experiment and a 6-week housing experiment of mice. EOJC was collected during SHS processing of cedar bedding and was administered orally to mice. Between days 1 and 2 after administration, hepatic P450s were significantly induced as shown in Figure 3. Histopathological examination showed the oral administration of essential oil caused lesion to hepatocytes and gastric mucosa of mice. The housing study of mice indicated that fresh cedar beddings increased the levels of these P450s in mouse liver, whereas mice housed in the improved cedar bedding showed significantly lower levels of P450s, indicating that SHS effectively removed those harmful organic compounds (volatile sesquiterpenes and naphthalene-derived aromatics) for animals from bedding. The housing study showed that SHS processing of

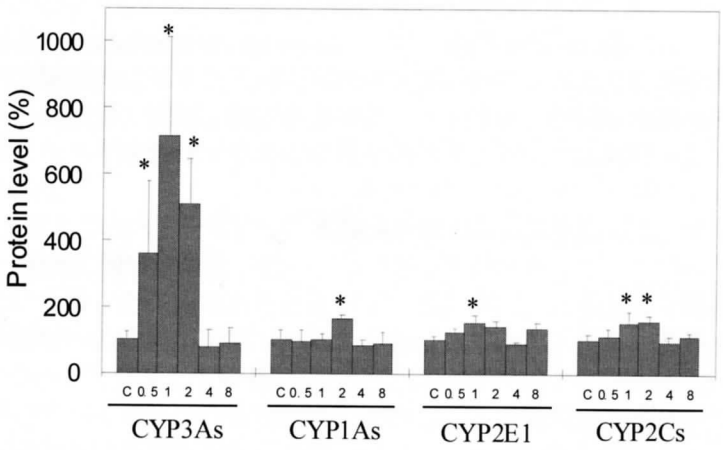


Figure 3 Hepatic P450 protein levels in mice after oral administration of EOJC. Male mice were treated with EOJC (2000 mg/kg) and killed at 0.5, 1, 2, 4, and 8 days. The value for control mice (C) was set at 100%. Data represent the mean ± S.D. (n = 5). * $P < 0.05$, vs. control mice.

bedding did not bring significant effects on the body weight, blood, the litter size at birth and weaning in mice, indicating no or minimal effect on the growth and reproductive capacity of mice. The improved cedar bedding was showed to be a kind of safe bedding for animal experiments to gain reliable data.

In chapter VI, a summary based on results and discussions from the previous chapters is provided. Superheated steam has been demonstrated to be an effective technique for the isolation of essential oil from plants. Superheated steam processing promoted the utilization of cedar resources and recycling cedar wastes, suggesting that superheated steam is a promising method on improving and recycling biomass wastes.

論文審査結果の要旨

水は、従来から反応媒体として研究されてきたが、過熱水蒸気についての既往の研究は多くなく、その性質ならびに応用できる範囲は未だによく分かっていない。過熱水蒸気とは、臨界点より低い温度、飽和蒸気圧未満の低圧力領域の水蒸気を指す。この温度圧力領域の水蒸気は、密度が低く、誘電率、イオン積も常温の水より低いという特徴を有し、有機反応を促進する反応場を与えると考えられている。

本論文は、スギの葉、端材や廃材を資源化することを研究目的としたものである。過熱水蒸気を利用してスギ資源から有価物質の抽出を行い、さらにスギ廃材を改質して実験動物用の床敷材としての有効利用を試みており、全6章で構成されている。

第一章“Introduction”では、スギ資源の活用が期待されている社会背景および反応媒体としての過熱水蒸気の特性を述べた。

第二章“Properties of superheated steam as an extraction medium”では、過熱水蒸気による流通型抽出装置を開発し、スギの葉を対象として、過熱水蒸気の抽出特性を調べた。反応条件として温度―水蒸気分圧を制御し次の結果を得た。すなわち、窒素あるいは飽和水蒸気による抽出と比較し、過熱水蒸気では対流伝熱および凝縮伝熱によって有機物質の抽出が促進されることを明らかにした。

第三章“Application of superheated steam to selective extraction of organic compounds from cedar leaves and utilization of extracts”では、過熱水蒸気によるスギの葉からの有機物の選択的抽出を行った。過熱水蒸気は、有機溶媒と比較してセスキテルペンおよびセスキテルペンアルコール類の抽出に有効であることを示した。セスキテルペン類は各種の生物活性に関与していると考えられており、注目すべき物質である。微生物学的見地から、スギの葉からの抽出物の生物活性成分の定性分析を行い、さらに、過熱水蒸気抽出物の優れた抗菌性を発見した。

第四章“Improvement of cedar bedding by using the superheated steam process”では、スギの実験動物床敷としての応用を提案し、過熱水蒸気処理の大型実用機によってスギ材の改質を行った。床敷に必要と考えられる木質構造を破壊することなく、実験動物の代謝に影響する揮発性のセスキテルペン類や芳香族炭化水素を選択的に抽出し除去することを試みた。その結果、過熱水蒸気処理によりスギ材からセスキテルペン、ナフタリン誘導体を効率的に除去し、床敷材に応用可能な程度にまで品質を向上させることができた。

第五章“Effects of the improved cedar bedding on laboratory animals”では、過熱水蒸気処理によって改質して得られたスギ床敷材について、実際の実験動物に対する使用の可能性を実験動物へ与える影響を調べることによって検討した。具体的には、マウスの成長、繁殖、臓器組織構造、血液性状、肝臓酵素誘導特性などに及ぼす影響を調べた。その結果、改質したスギ床敷はマウスの成長を妨げず、安全な床敷材として飼育に利用することが可能であることが確認された。

第六章“Summary”では、第二章から第五章までの内容を要約し、本論文を次のように総括した。すなわち、200℃以下の過熱水蒸気を用いた反応は、環境に対する負荷が低く、低コスト・低エネルギーといった利点があるとし、過熱水蒸気は環境保全の理念に合致した技術であると述べている。次に、日本全国で過多とされるスギ材の活用を目指し、小動物用の床敷材への応用が可能であることを示している。また、過熱水蒸気によるバイオマス未利用資源の有効活用についても言及している。

以上のように、本論文は、過熱水蒸気を用いてバイオマスの有効利用を検討し、応用展開の一例を示したものである。その研究内容は、工学・医学・薬学という多方面から学際的にアプローチしたものであり、今後の環境科学の発展に寄与するところが大きい。

よって、本論文は博士(学術)の学位論文として合格と認める。