GREEN SOLVENT PROCESSING PLATFORM FOR THE EXTRACTION OF THE NUTRACEUTICAL INGREDIENTS FROM UNUSED BIOMASS - CITRUS FRUITS -

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ABSTRACT

A platform is proposed for the extraction of nutraceuticals ingredients from natural products, which includes the following three processes:

1. Single-stage extraction using scCO_2 (supercritical CO_2).
2. Single-stage extraction using of high-pressure hot water and/or hot water with scCO_2.
3. Multistage extraction, with counter-currently contact between scCO_2 and H_2O with the raw materials in the extractor (Hybrid SFE).

Components extracted from plant materials, such as lipids, saccharides, and glycosides, are valuable for medical applications. High-pressure hot water complements scCO_2 for environmentally benign processing of nutraceuticals. This “green” solvent processing platform offers an “all-natural” approach to processing natural products for nutraceutical ingredients. This paper describes the treatment of citrus fruit waste using the “green” solvent processing platform for unused biomass. *Citrus junos*, called yuzu in Japan, is processed into juice and is often preferred to vinegar. After juice processing (cold pressing), a large amount of fruit pulp (mainly composed of peel) is considered waste and incurs a cost for disposal. The nonpolar components of *Citrus junos*, such as terpenoids and carotenoids, are preferentially removed by CO_2 followed by extraction with a CO_2-cosolvent combination that can remove the more polar components, such as flavonoids or coumarines. In this stage, the hybrid SFE system is useful for the separation of flavonoids. The separation can be controlled by adjusting the CO_2 and water in the extraction field; therefore, the hybrid process can extract hydrophilic and hydrophobic components at the same operation stage. The hybrid SFE process is useful for the separation of hydrophilic flavonoids (hesperidin) and hydrophobic flavonoids (tangeretine, nobiretine) from waste citrus residue. After removal of the flavonoid components, subcritical water can be applied to isolate saccharides, followed by other compounds.

INTRODUCTION

Medical expenditures in Japan have increased dramatically due to the aging of its citizens. Along with this societal change, the market for health-related food and supplements was 1.6 trillion Japanese yen in 2005; 3.0 trillion yen is expected in 2010. The nutraceutical market was about 680 billion yen in 2007, as shown in **Figure 1**[1)]. The Japanese Ministry of Health, Labor, and Welfare reviewed the system supplying health foods and supplements in Feb. 2005. In addition, the increasing need to ensure the safety of food and supplements led to regulations concerning pesticide residues in food in May 2006.

The application of supercritical fluid extraction (SFE) as a safe procedure in the food and nutraceutical industries is gaining momentum in Japan. Nutraceutical ingredients from plant materials, such as lipids, saccharides, and glycosides, are valuable target materials of the extraction.

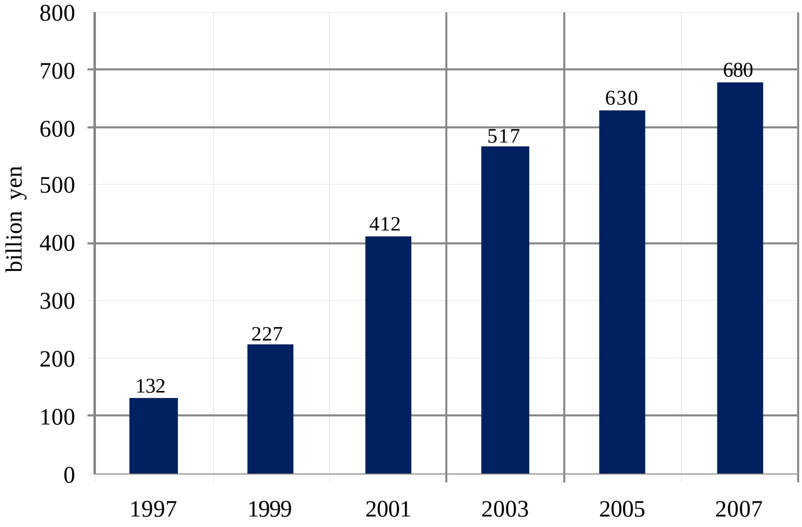

Figure 1 Nutraceutical Market in Japan

The quantity of generation of the food wastes is about 22 million tons as the biomass resources in Japan (total quantity of generation in 2005 is 212.3 million tons). About 80% of the food waste is unused as incineration or reclamation, though about 20% is used for the compost or animal fodder (**Figure 2**). The research basis plan of the Japanese Ministry of Agriculture, Forestry and Fisheries has pointed the production of the materials of fine food such as the functional food from the waste generated from the agriculture, forestry and fisheries industry and the foods industry is an important theme.

Under such background, the market needs for the green solvent extraction concerning the production of functional food materials from food wastes, is increasing. Goto's research group (Kumamoto University) has targeted to fabricate the basic structure of the green solvent processing platform for corresponding to these market need. Some experimental data for the green solvent extraction of the nutraceutical ingredients from unused citrus fruits are shown in this paper.

Biomass	Quantity of Generation (1000ton)	Use and application state	Quantity of Unused (1000ton)
Livestock wastes	89,000	Compost (90%) / Unused (10%)	8,900
Food waste	22,000	Compost and animal food (20%) / Unused (80%)	17,600
Sawmill waste	5,000	Fuel and compost (90%) / Unused (10%)	500
Construction waste	4,600	Paper stock and animal bedding (60%) / Unused (40%)	1,840
Sewage sludge	75,000	Building materials and compost (64%) / Unused (36%)	27,000
Forestry waste	3,700	Almost unused (100%)	3,700
Agricultural produce	13,000	Compost, animal food /bedding (30%) / Unused (70%)	9,100
Total	212,300		68,640

Figure 2 Quantity of Biomass Generation and its Use in Japan (2005)

GENERAL DESCRIPTION OF GREEN SOLVENT PROCESSING PLATFORM

Subcritical water complements supercritical CO_2 ($scCO_2$) for the environmentally benign processing of nutraceuticals. **Figure 3** shows an "all-natural" approach to obtaining nutraceutical ingredients, which is considered a "green" supercritical fluid process. The nonpolar components of citrus waste, such as essential oils, carotenoids, triterpenes, and phytosterols, are preferentially extracted by CO_2 followed by treatment with a CO_2-water combination that can extract the more polar components, such as flavonoids. Finally, after removal of the initial components, subcritical water can be applied to isolate polysaccharides and other compounds.

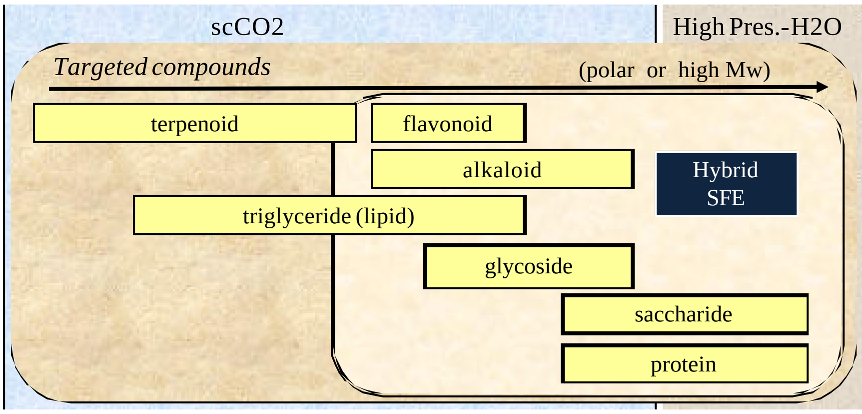

Figure 3 Green Solvent Processing Platform

Single stage extraction using $scCO_2$

SFE can be used in several modes for extracting nutraceutical ingredients or functional foods. Extraction with scCO2 or a scCO2-cosolvent mixture can be used to yield an extract equivalent to that obtained from organic solvent extraction. **Table 1**[3)] lists some common and popular nutraceutical ingredients in use today, their application, and the use of SFE for their production.

Many active ingredients from plant materials and other natural materials cannot be easily extracted using SFE at pressures below about 30MPa. A cosolvent is needed, as well the addition of a step to remove the cosolvent from the product. The solubility of $scCO_2$ for low-soluble substances increases at high pressure. The enhanced solubility in $scCO_2$ at high pressure allows it to act as a cosolvent for low-soluble substances (**Figure 4**).

Table 1 Nutraceuticals and Their Therapeutic Use and Current Productin by SFE

Nutraceuticals	USES	Processed by SFE
Saw palmetto	Prostate	Commercial
Kava-kava	Anxiolytic	Not commercial
Hawthorne	Cardiotonic	Not commercial
Ginseng	Tonic	Commercial
Garlic	Circulatory	Commercial
Ginko biloba	Cognitive	Not commercial
St.John's wort	Depression	Not commercial
Chamomile	Dermatological	Commercial
Echinacea	Colds / flu	Commercial
Black cohosh	Gynecological	Not commercial
Lutein	Macular degeneration	Commercial
Flavonoids	Anticancer	Not commercial
Isoflavones	Premenstral syndrome, circulatory	Not commercial
Marine oil fatty acids	Circulatory	Commercial
Evening primose	Inflammation	Commercial
Phytosterols	Circulatory	Commercial
Tocopherols	Antioxidant	Commercial
Phospholipids	Cognitive	Commercial

Single-stage extraction using high-pressure hot water and/or hot water with $scCO_2$

Water, in its supercritical region (scH_2O) and in its subcritical region, is an alternative to organic solvents. Water has the capability of dissolving a variety of organic solutes, because its dielectric constant (permittivity) can be adjusted as a function of temperature. Under appropriate conditions, pressurized water can solubilize even the most nonpolar, hydrophobic organic compounds. The conversion of waste biomass to energy resources has been widely studied. **Figure 5** shows the protocol for biomass processing in water. Interest in pressurized hot water (superheated water as liquid water under pressure between 100°C and 374°C) has been renewed as a "green" substitute for organic solvents in separations and related processes. Many extraction processes are restricted to the temperature range of 100°C to 300°C[4]. At these lower temperatures, water is not highly compressible, and the pressure of the system does not exert much effect, as long as it is high enough to maintain the water in the liquid phase.

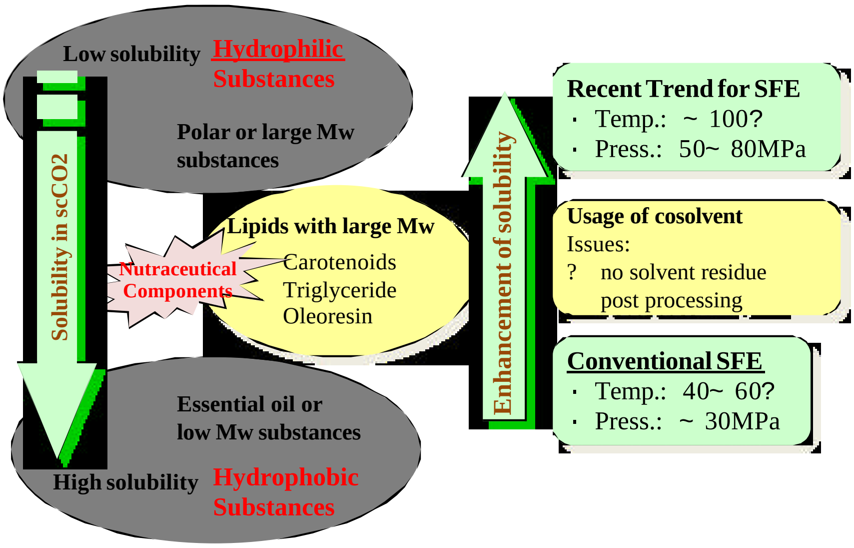

Figure 4 SFE Processing

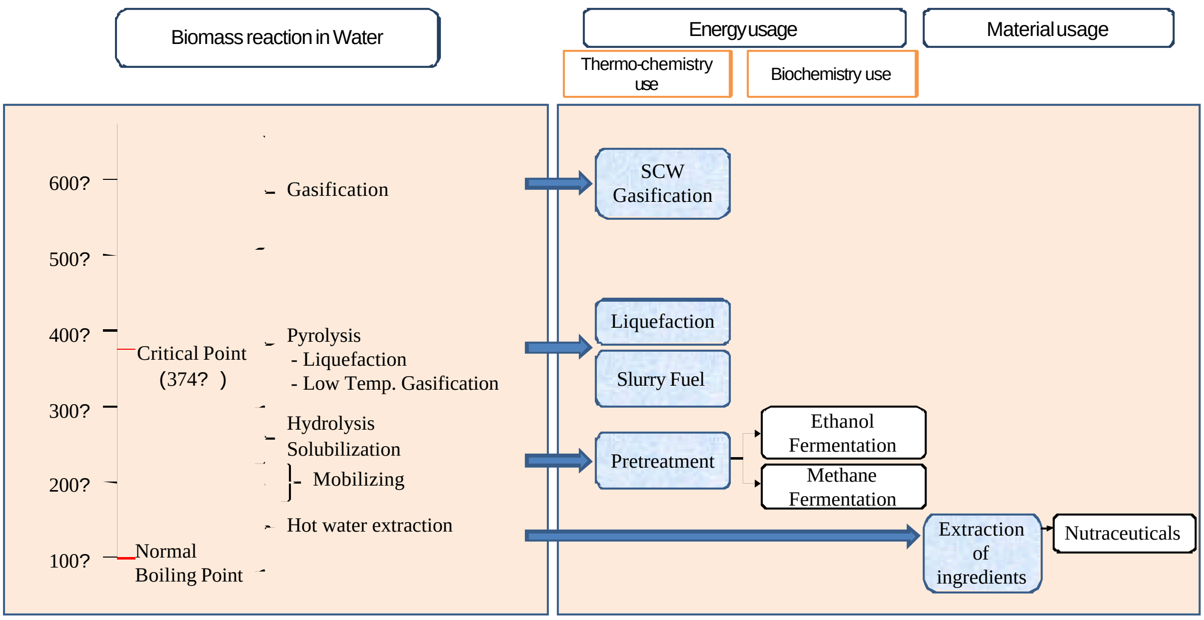

Figure 5 Protocol for Biomass Processing in Water

The polypore fungus *Ganoderma lucidum* provides bioactive compounds, including several triterpenoids and polysaccharides that are reported to possess anti-cancerous and immunomodulatory properties. The extraction yield of polysaccharides from *Ganoderma lucidum* is *ca*. 80% at 473°K. At temperatures higher than about 500°K, the extraction yield decreases, due to degradation and polymerization[5]. Pectin also has been separated from the flavedo of *Citrus junos*[6]. The relation between optimum extraction temperature and molecular weight of an extracted polysaccharide is shown in **Figure 6**, and is based on experimental extraction results from *Citrus junos* and *Ganoderma lucidum*. For the extraction of pectin from *Citrus junos*, extraction time can be shortened by 10% compared with a conventional extraction method such as acid solution extraction. For *Ganoderma lucidum*, extraction yield can be increased approximately 10-fold compared to the conventional method.

Hybrid SFE [7]

Goto's research group (Kumamoto University) has developed a hybrid SFE process that uses CO_2 and water as solvents. For the hybrid SFE, CO_2 and water are placed in contact with the raw materials in the extractor counter-currently, as shown in **Figures 7** and **8**.

The hybrid SFE process can use $scCO_2$ as the dispersion phase and can also use as a continuous phase during operation. In both cases, $scCO_2$ and hot water are placed in contact with the raw materials in the extractor, counter-currently. Hydrophilic components are extracted by hot water from the raw material, and hydrophobic components are extracted by scCO2 at the same stage. Separation factor of each component can be controlled by adjusting flow rate of CO_2 and water in the extraction field. Therefore, the hybrid process is able to extract hydrophilic components and hydrophobic components during the same

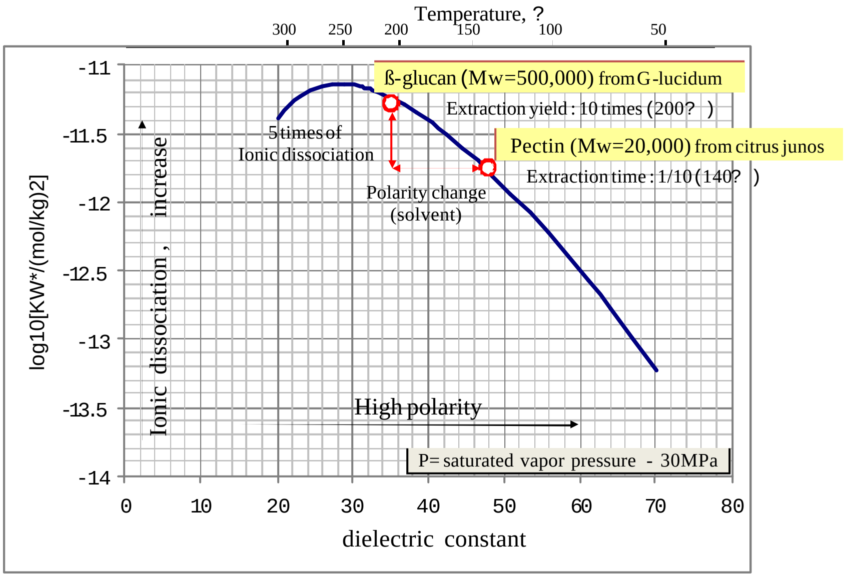

Figure 6 Relation between Optimum Extraction Temperature and Mw of Polysaccharide in Raw Material

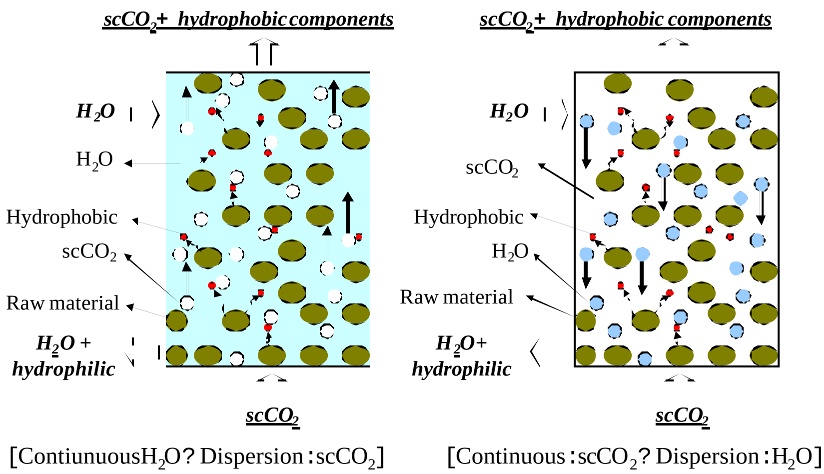

Figure 7 Extraction Model of Hybrid SFE

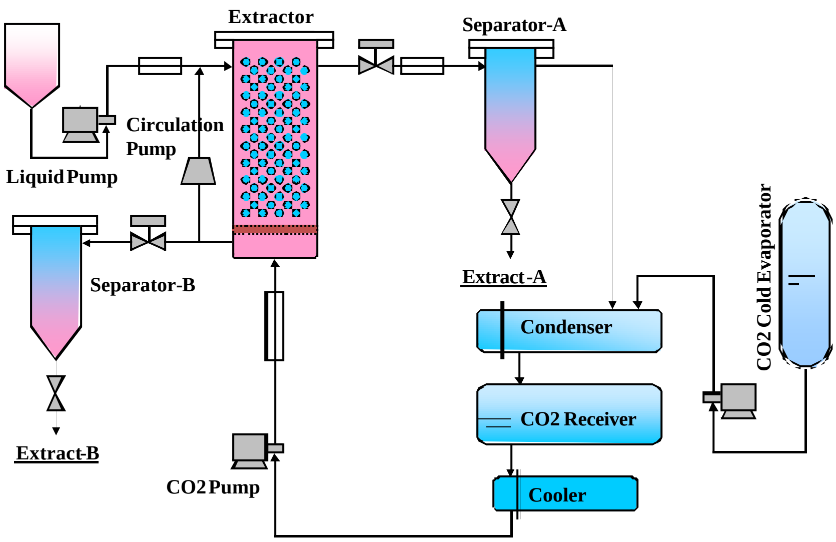

Figure 8 Process Flow of Hybrid SFE

operation. The results confirm that the hybrid SFE process is useful for the separation of ginseng extracts and pesticides from ginseng roots. This method is also effective for the separation of caffeine and polyphenols from green coffee beans in high yield.

GREEN SOLVENT PROCESSING PLATFORM FOR UNUSED CITRUS FRUITS

Citrus Junos, called yuzu in Japan, is a popular citrus fruit in Japan and other east Asian countries. It is used in many food products such as salads, sauces, dressings, and vinegar because of its desirable flavor. It is also used as an ingredient in bathing products. Yuzu essential oil has a characteristic flavor that is stronger than that of other citrus fruits. The yuzu essential oil is of

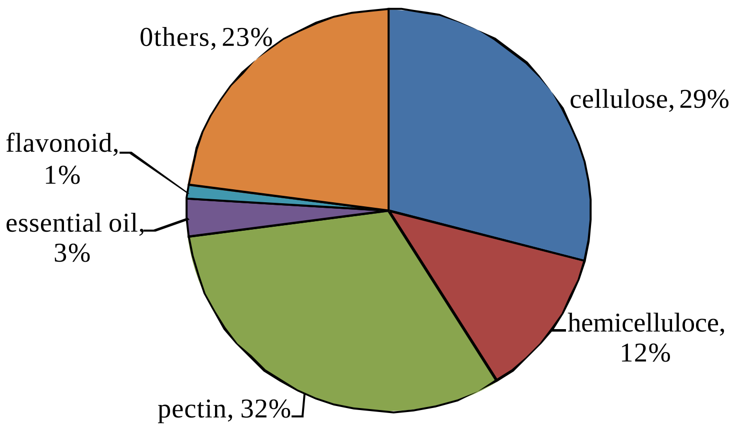

Figure 9 Composition of Cold-pressing Residue of *Citrus Junos*

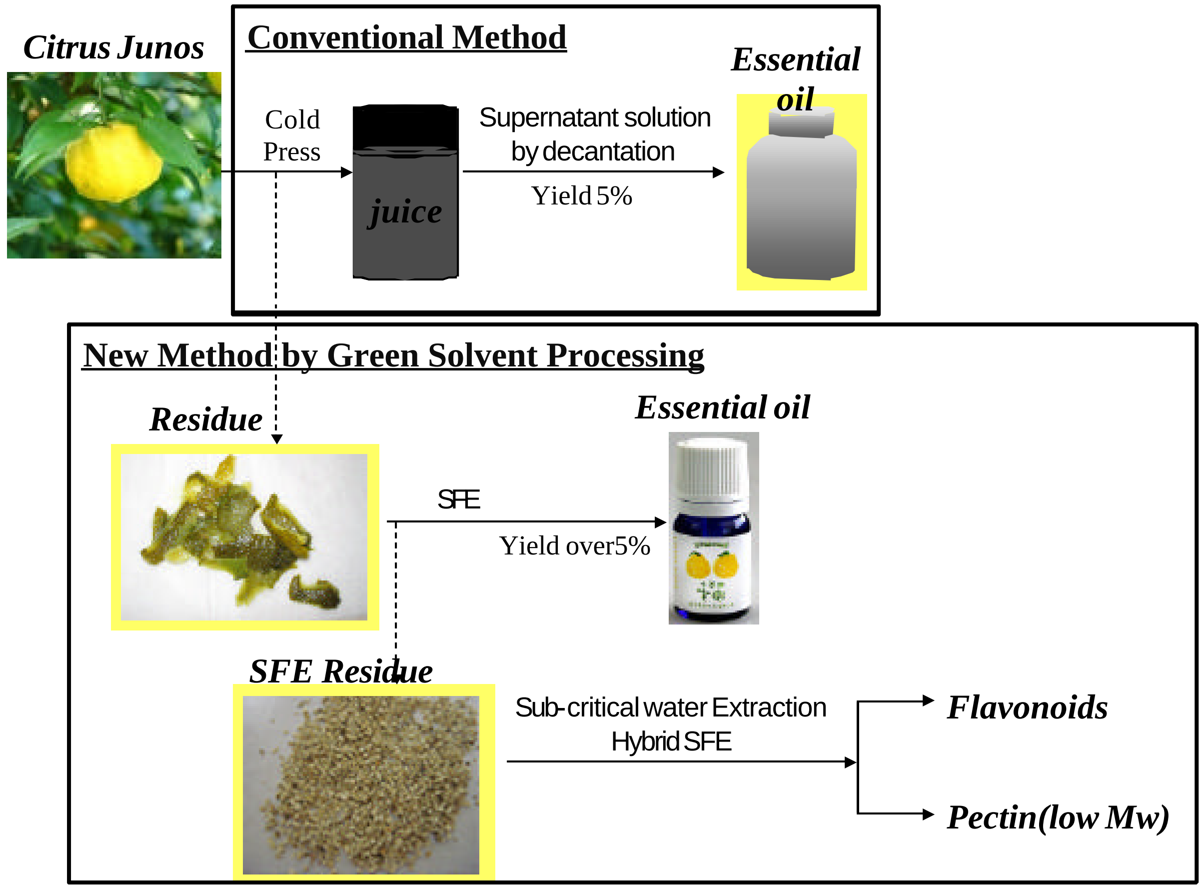

Figure 10 Concept of Green Solvent Processing for Citrus Fruit Industry (*Citrus Junos*)

interest for aromatherapy. The conventional production process for citrus juice that includes the essential oil is cold press. The essential oil is recovered by decantation after the cold press. The percentage of cold press residue (mainly composed of peel) from original *Citrus junos* fruit is *ca*. 80%. However, this waste residue contains many nutraceutical ingredients, such as terpenoids (also included in the essential oil), flavonoids, and pectin (**Figure 9**), and incurs costs for disposal.

The concept of green solvent processing for the citrus fruit industry is shown in **Figure 10**. For *Citrus junos*, nonpolar components, such as terpenoids and carotenoids, are preferentially extracted by CO_2 followed by extraction with a CO_2-cosolvent combination that can extract the more polar components, such as flavonoids or coumarines. In this stage a hybrid SFE is useful for the separation of flavonoids. Separation factor of each component can be controlled by adjusting the flow rates of CO_2 and water in the extraction field; therefore, the hybrid process is can extract hydrophilic and hydrophobic components during the same operation. The hybrid SFE process was shown to be useful for the separation of hydrophilic flavonoids (hesperidin) and hydrophobic flavonoids (tangeretine, nobiretine) from waste citrus residue. And, after removal of the flavonoids, subcritical water can be applied to isolate the saccharides followed by other compounds.

$ScCO_2$ extraction of waste citrus fruit biomass

SFE has been applied to the extraction of essential oils from unused citrus fruit residue remaining after cold pressing. In this study, the peel of *Citrus junos* was used as the raw material. The experimental results at 80°C and 30 MPa for a batch operation and a semi-continuous operation are shown in **Figure 11**. The extraction yield of essential oil was 92% at *ca*. 30 of S/F(Solvent ratio to Feed) from the semi-continuous operation. A semi-continuous operation is the conventional method for SFE during which $scCO_2$ is continuously supplied to the extractor where the raw material is charged. In contrast, an extremely high yield of *ca*. 100% was obtained from the batch-wise operation (pressure swing operation) at

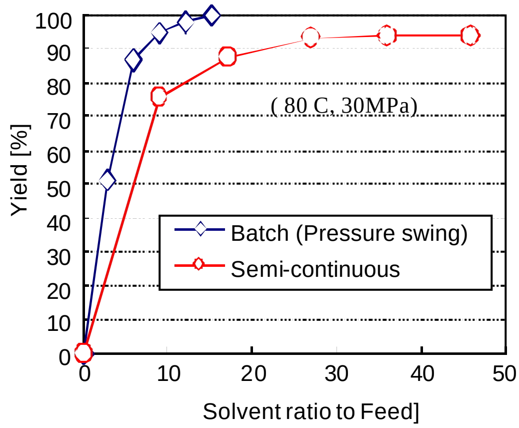

Figure 11 Experimental Results of SFE

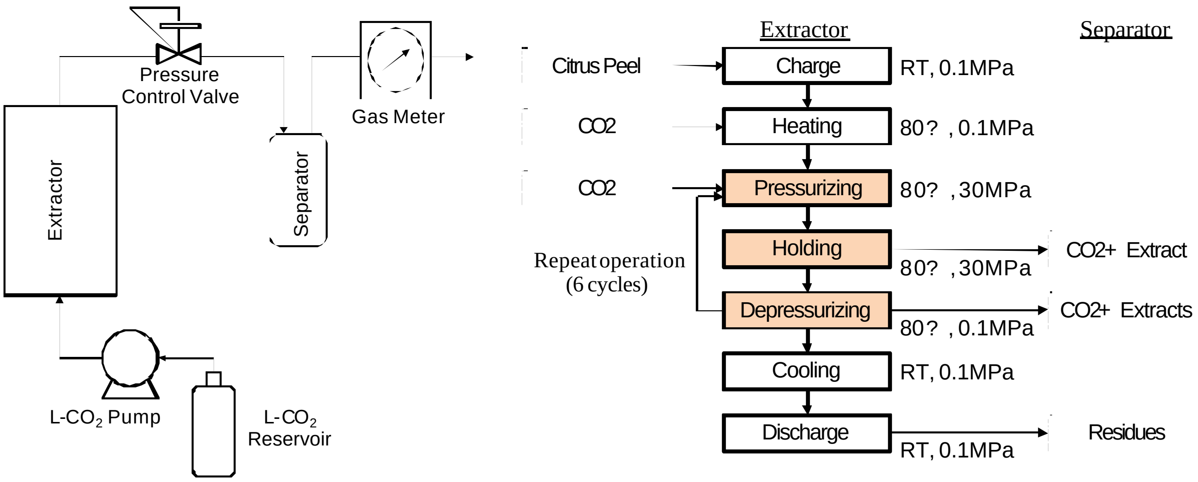

Figure 12 Process Flow of Experimental Apparatus and its Operation Mode of Pressure-swing SFE

ca. 15 of S/F. **Figure 12** shows the pressure swing during the batch operation, referring to the process flow chart of the experimental SFE apparatus. The raw material is charged to the extractor. Then, the extractor is adjusted to the desired temperature and pressure. After the extractor reaches the desired conditions, they are maintained for a fixed period. One hour is desirable as the hold time based on experimental results. After maintaining the desired conditions for one hour, the extractor is depressurized. This operation is the first extraction. For the pressure swing operation, this cycle is repeated several times.

Hybrid SFE process for waste citrus fruits

Hybrid SFE was applied to the extraction of the separation of hydrophilic flavonoids (hesperidin) and hydrophobic flavono**e**ids (tangeretine, nobiretine) from waste citrus residue.

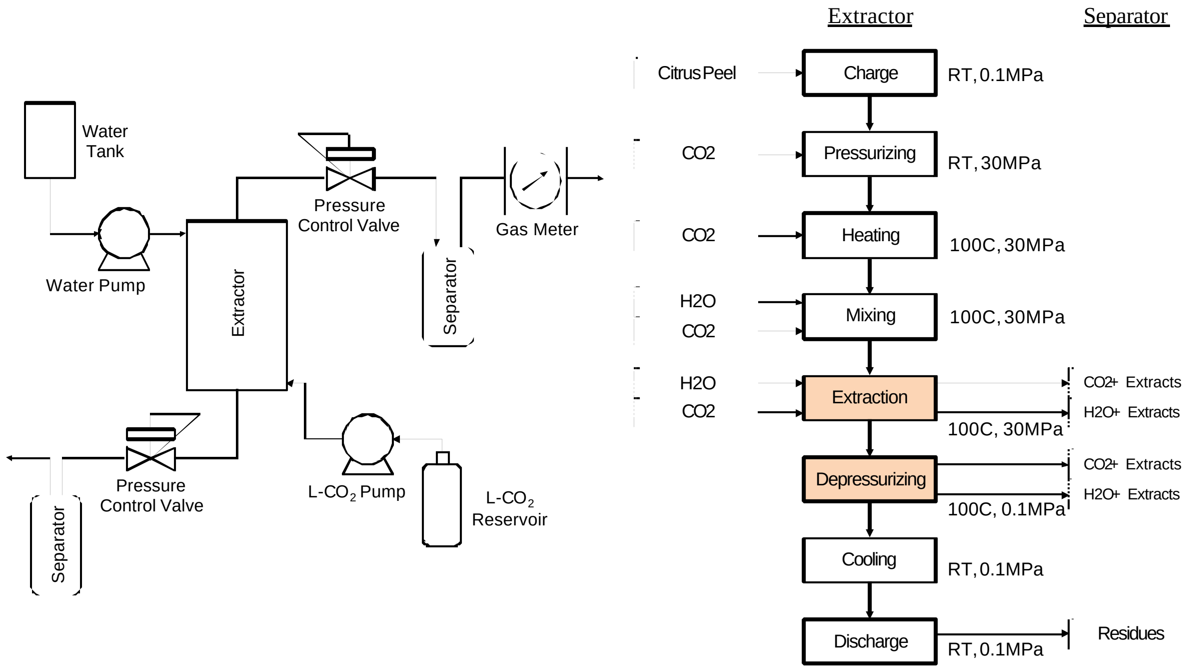

Figure 13 Process Flow of Experimental Apparatus and its Operation Mode of Hybrid SFE

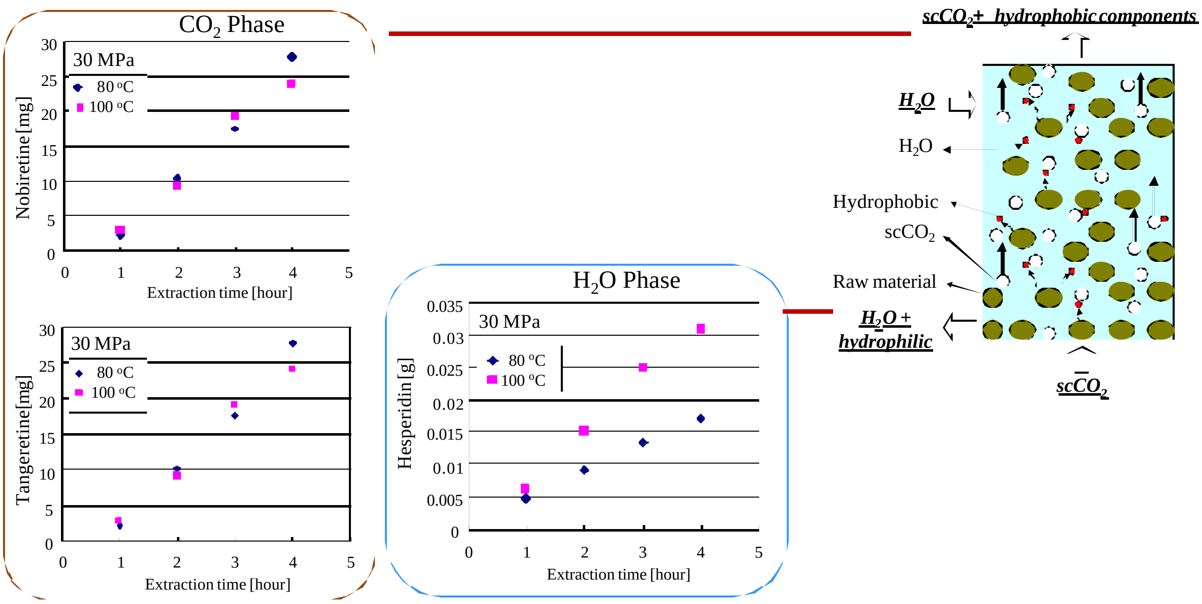

Figure 14 Experimental Results of Hybrid SFE

Figure 13 shows the operation mode of the hybrid SFE process, referring to the process flow chart of the experimental SFE apparatus. The peel of *Citrus junos* was used as the raw material, and was added into the extractor. Then, the extractor was pressurized at the desired temperature and pressure by supplying CO_2 from the bottom of the extractor. After the extractor reaches the desired conditions, water is supplied to the extractor from the top. $ScCO_2$ and water are allowed to contact the raw material in the extractor, counter-currently.

Figure 14 shows experimental results from the hybrid SFE. Hesperidin, which has a glycoside group and so is hydrophilic, is extracted into the water phase. Nobiretine and tangeretine, which are low polarity because they possess methyl groups and so are hydrophobic, are extracted into the CO_2 phase.

Pressurized hot water extraction

Figure 15 shows the effect of pressure on the extraction yield of pectin using subcritical water with CO_2 at the experimental temperature of 120°C (volume ratio of water to CO_2 was 3:1). The control in this figure is the experimental result without CO_2 at 120°C and 30 MPa. When CO_2 is added to the subcritical water, the extraction yield of pectin increased with pressure, and produced a maximum yield of 92%.

Figure 16 shows the change in molecular weight of pectin with extraction pressure. The average molecular weight of the extracted pectin was *ca.* 20,000. In contrast, addition of CO_2 caused the molecular weight of extracted pectin to decrease with increasing pressure. Water in contact with CO_2 becomes acidic due to the formation and dissociation of carbonic acid. The solubility of CO_2 in water at a subcritical state increases with pressure. Therefore, the decrease in molecular weight with the rise in pressure is caused by the hydrolytic reaction of the pectin due to the effect of the acid catalyst. Thus, the hydrolysis of pectin is enhanced by increasing the concentration of carbonic acid in subcritical water.

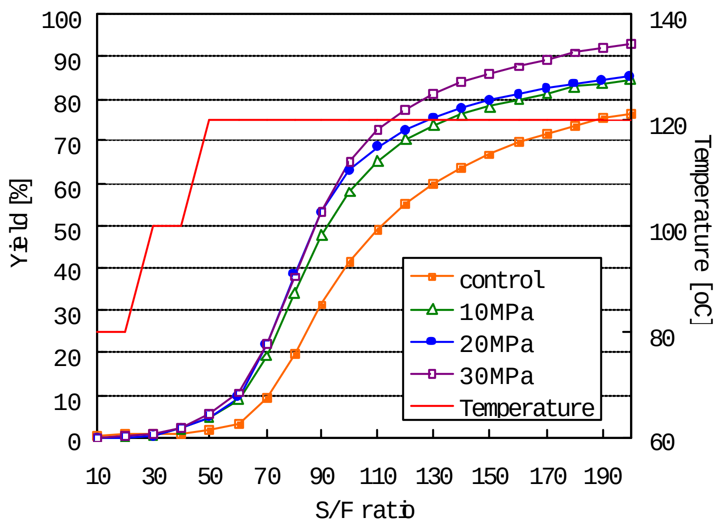

Figure 15 Experimental Results of Pectin Extraction with pressurized hot water (120C)

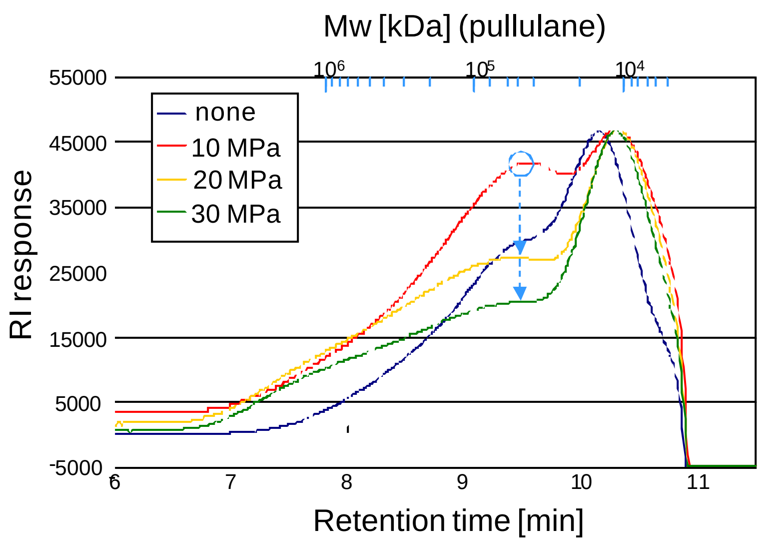

Figure 16 Effect of Pressure on the Molecular Weight of Pectin under adding of pressurized CO_2

CONCLUSIONS

Because a supercritical carbon dioxide and water combination is an environmentally benign solvent, and possesses excellent solvent characteristics, it is utilized in many fields. SCF technologies (extraction and fractionation) have been applied to the study of nutraceutical ingredients for more than 30 years. The extraction is a core technology of supercritical fluid technology.

The green solvent extraction platform described here indicates the direction of the supercritical fluid extraction technology into the future. Fine particle production and food material modifications through the use of supercritical fluid technology may join other generic processing methods. A "green" processing platform involving the use of environmentally benign fluids and combined unit processing applications is emerging. Such an integrated supercritical fluid processing platform will make maximum use of commercial-scale production, and will include processes for particle production, tailored toward the nutraceutical marketplace. In addition, it will effectively use agricultural and fishery waste as the raw materials, with the final residue remaining after extracting all desired compounds decomposed by supercritical water oxidation (SCWO).

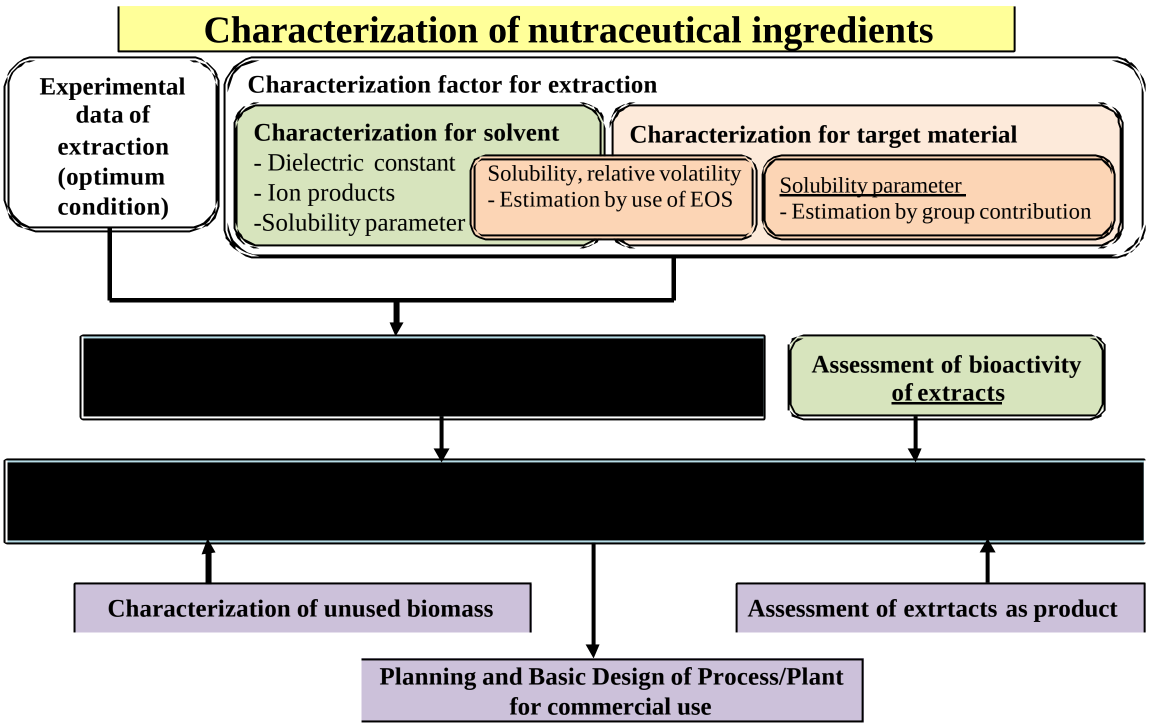

Figure 17 Role of the Green Solvent Processing Platform

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