Utilization of citrus peel by sub- and supercritical fluid technology
Motonobu Goto\textsuperscript{a}, Siti Machmudah\textsuperscript{a}, Mitsuru Sasaki\textsuperscript{b}, Masahiro Tanaka\textsuperscript{c}

\textsuperscript{a} Bioelectrics Research Center, Kumamoto University, Kumamoto, Japan (mgoto@kumamoto-u.ac.jp)
\textsuperscript{b} Graduate School of Science and Technology, Kumamoto University, Kumamoto, Japan
\textsuperscript{c} Ascii Co. Ltd., Fukuoka, Japan

ABSTRACT
In the citrus juice production process, a large amount of by-product is generated. We have been developing effective utilization processes to produce high value products by using sub- and supercritical fluids. Supercritical CO\textsubscript{2} was used as a green solvent to extract components such as essential oil, flavonoids, oil from citrus peel and seeds. Subcritical water was used for reactive extraction of pectin and flavonoids from citrus peel. We also developed a hybrid process to use supercritical CO\textsubscript{2} and subcritical water for the efficient extraction process. In this work, yuzu (\textit{Citrus junos}) was used as a citrus fruit. Essential oil was extracted with supercritical CO\textsubscript{2} from dry or wet citrus peel. Since yuzu contains a large amount of seed, supercritical CO\textsubscript{2} was used to extract N-methylanthranyl acid methyl, fatty acid, \(\beta\)-sitosterol, squalene, and vitamin E, as well as seed oil. Fractionation using a countercurrent extraction tower is a promising process for deterpenation where aroma components of citrus oil is concentrated. Essential oil obtained from yuzu peel was fractionated and oxygenated and sesquiterpene components are enriched. By hydrothermal extraction where subcritical water was flowed through a packed bed of citrus peel after extraction of essential oil, hydrophilic component, pectin, and minerals were eluted. As the temperature was increased step-wise up to 200°C, pectin was extracted accompanied by molecular weight reduction. Hybrid extraction was carried out using various type of extractor. By the mixed flow of supercritical carbon dioxide and water, hydrophobic components were selectively extracted into carbon dioxide phase, whereas hydrophobic components were extracted into water phase. By increasing the process temperature, pectin was also extracted.

Keywords: Citrus fruit; supercritical carbon dioxide; subcritical water; extraction

INTRODUCTION
Citrus fruits have been widely used to produce juice and the other food products. In the juice production process, a large number of wastes are generated. Since the waste contains valuable materials, they should be utilized to produce high-value products. In this paper, the development of extraction or reactive-extraction technologies using supercritical fluid for the treatment of citrus waste is described. The objective of this research is to develop high pressure technology to produce valuable materials from by-product of citrus juice processing as shown in Fig. 1. By using supercritical carbon dioxide, essential oil and pigments were extracted from the citrus peel. After that process, subcritical water was used to extract pectin and the flavonoids. Various citrus fruits were used as the raw materials. We also proposed hybrid process using simultaneously supercritical carbon dioxide and water to extract hydrophobic and hydrophilic compounds from citrus peel.

MATERIALS & METHODS
Yuzu (\textit{Citrus junos}) was used as a citrus fruit. Citrus peel and citrus juice production residue were used. A semi-batch supercritical CO\textsubscript{2} extraction apparatus (extractor: 500 ml,
AKICO Co., Japan) was used to extract essential oil and pigment. Fractionation of citrus essential oil was carried out by a countercurrent extractor with a column of 20 mm i.d. and 1.8 m long packed with Dixon Packing Subcritical water extraction was carried out (Toyo Koatsu Co., Japan). Subcritical water extraction from citrus peel after supercritical CO$_2$ extraction was carried out by a semi-batch extractor (8.7 mm i.d., 118 mm long). A hybrid extraction apparatus consists of an extractor with cocorrent or countercurrent flow of supercritical CO$_2$ and water through packed bed of citrus peel.

RESULTS & DISCUSSION

Supercritical CO$_2$ extraction from citrus peel
Essential oil was extracted with supercritical CO$_2$ at temperatures of 40 – 60 °C and pressures of 10 – 30 MPa. The highest extraction yield of 91 % was obtained at 40 °C and 10 MPa. The monoterpane content in the extract was 89.9 % and oxygenated compounds were 7.7 %.

Supercritical CO$_2$ extraction from citrus seed
Since the fraction of seed in yuzu is considerable, utilization of the seed is important. Supercritical CO$_2$ extraction from ground seed was carried out at temperatures of 40 – 70 °C and pressures of 20 – 50 MPa. The highest extraction yield was obtained at higher pressure and temperature (50 MPa and 70 °C). The main components in the extracts were N-methylanthranyl acid methyl as volatile compound, fatty acid, β-sitosterol, squalene, and vitamin E as shown in Fig. 2.

The fatty acids in the oil extracted using supercritical CO$_2$ are palmitic, linoleic and oleic acid as major components and the minor compounds are palmitoleic, stearic and 10-octadecenoic acid.

Fractionation of citrus essential oil using countercurrent extractor with supercritical CO$_2$
For a semi-batch operation of yuzu oil was carried out at conditions of temperatures of 40 – 100°C and pressures of 8.8 – 20 MPa. Enrichment of oxygenated compounds and sesquiterpene is the objective of the fractionation. In yuzu oil, typical characteristic aroma compounds are linalool and β- and δ-elemene. As terpenes are extracted selectively, these components are

![Figure 2. Extracted compounds from seed at 343K and 20 MPa](image2)

![Figure 3. Effect of temperature on oxygenated compounds content of the fractions and residue obtained by SCCO$_2$ column fractionation at 8.8 MPa](image3)

![Figure 4. Hydrothermal extraction behavior of pectin (galacturonic acid) and cations](image4)
enriched in later fraction or residue as shown in Fig. 3. High recovery of these aroma components in the residue was obtained at the extraction at 60 °C. Temperature gradient along the extraction column improved the separation. Higher enrichment of sesquiterpene could be obtained at 8.8 MPa with temperature gradient of 40 – 100 °C. The higher yield of extract was found at 40 °C. 94% of δ-elemene was recovered at 88 MPa and 60 °C.

Subcritical water extraction
Pectin was separated from the yuzu flavedo with subcritical water using a semi-continuous extractor. Water was continuously delivered into the extractor while gradually increasing the temperature from 60 to 160 °C at 20 MPa. Pectin was rapidly extracted when the temperature of the extractor reached 160 °C as shown in Fig. 4. The yield of pectin reached as high as 80%. Large amount of potassium was extracted just prior to the extraction of pectin, because extraction of potassium indicates destruction of the cell wall and membrane. Calcium and magnesium was also extracted.

Hybrid extraction with supercritical CO$_2$ and water
We have proposed a hybrid extraction process with supercritical CO$_2$ and water. As shown in Fig. 5, supercritical CO$_2$ and water are flowing counter-currently over a packed bed of solid sample. Hydrophobic and hydrophilic compounds are selectively extracted into CO$_2$ and water phase, respectively. Extraction of pectin and flavonoids were extracted by using a semi-continuous flow extractor with a mixed flow of subcritical water and supercritical CO$_2$ at temperatures of 40 – 120 °C and pressures of 10 – 30 MPa. Increasing pressure accelerated pectin extraction and dissolved CO$_2$ showed acidic effect. 90% of pectin was obtained in water phase at 120 °C and 30 MPa as shown in Fig. 6. Supercritical CO$_2$ with small amount of dissolved water was suitable solvent for extracting weakly polar flavones such as naringin and hesperidin.

CONCLUSION
By using supercritical CO$_2$ and subcritical water, wide spectrum of components from essential oil to pectin. CO$_2$ and water were used separately or simultaneously as a mixed solvent. Synergetic effect of CO$_2$ and water was observed.

REFERENCES

Figure 5. Concept of simultaneous extraction and separation process. = Water; = CO$_2$; = polar compound; = non-polar compound

Figure 6. Extracted pectin with subcritical water and supercritical CO$_2$ (water/CO$_2$ = 3/1) at 120°C and 30 MPa.
