論文の内容の要旨

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Characterization of *Citrus natsudaidai* peel and its application as a natural food additive

（柑橘類ナツミカンの皮の特性評価と天然食品添加物としての応用に関する研究）

<Chapter 1. Introduction>

Reducing food waste is a global challenge that significantly impacts the environment, society, and economics. At the United Nations Summit in 2015, the Sustainable Development Goals (SDGs) are adapted. SDGs Goal number 12 contains 11 targets. A target of 12.3 is set to "By 2030, halve per capita global food waste at the retail and consumer levels and reduce food losses along production and supply chains, including post-harvest losses". The concept of this study is to use up food processing waste using food-industrially available methods. It can estimate that the amount of plant food waste is about 8.36 million tons per year in Japan, and utilization of this waste effectively may greatly contribute to the economy and the environment.

In this study, citrus was selected as a sample because it is widely cultivated worldwide, its waste part ratio is high, and its processing waste can be eaten without heating. Among various citrus species, *Citrus natsudaidai* (CN) was used as a sample, assuming utilization of the residue after processing into food products such as juice and jelly. CN has a singular characteristic which is a very thick and hard peel. Thus, it is challenging to manage effectively; however, alternatives for its utilization should be considered to contribute to a reduction in waste.

The aim of this study was the utilization of food processing waste. The study was divided into three steps as follows: (1) investigation of nutritional and health-promoting components in the target food processing waste; (2) the establishment of extraction and separation method for components to be used; (3) a trial test assuming the actual utilization way.

<Chapter 2>

The color, proximate composition, free amino acids, fatty acids, minerals, and aroma compounds of three cultivars of CN peels were determined. Regarding the proximate composition and minerals, CN peels included approximately 18% of carbohydrate, which may consist of pectin, and the most predominant mineral was potassium. The color of peels varied, probably due to the difference in cultivars. The contents of free amino acids were not enough to affect the taste of extracts. The proportion of individual fatty acids was not comparable to other plant oils; however, typical aroma compounds with citrusy smell were identified. It was estimated that CN peel would be useful as an ingredient for pectin and natural flavor due to the carbohydrates and aromatic compounds.

<Chapter 3>

The purposes of this chapter were obtaining health-beneficial compounds from CN peels by simple and food-use extraction way; investigating the antioxidant capacities of extracts of different polarities without overestimating the results. CN peels were extracted with water-ethanol and hexane-ethanol mixture solutions to obtain five different soluble fractions. The highest total phenolic content (TPC) was obtained from a water-soluble fraction (WSF), and the lowest content was observed in hexane soluble fraction (HSF). WSF also showed the most active antioxidant activities, and minimum activities were found in HSF. A positive correlation was detected between TPC and antioxidant activities.

<Chapter 4>

In Chapter 3, CN peel extracts revealed the presence of phenolic compounds. There was a positive correlation between the TPC and antioxidant activity, meaning that the polar phenolic compounds are responsible for the antioxidant capacity of CN peels. Some of the flavonoids were identified in CN peel extract using the on-line HPLC-ABTS system, yet their antioxidant properties were not detected. In addition, there was a considerable difference between total antioxidant activity in the on-line HPLC-ABTS and ABTS recorded by spectrophotometer without compound separation in Chapter 3. Although many studies have used an on-line HPLC-antioxidant assay, the antioxidant capacity differs depending on the experimental conditions. Therefore, this chapter aimed to identify phenolic compounds acting as a radical scavenger in CN peel and improve an on-line HPLC-ABTS method simultaneously. The results verified that neoeriocitrin, narirutin, naringin, hesperidin, and neohesperidin were included in CN peel. Regarding the optimized experimental conditions to improve the ABTS reactivity of the on-line HPLC-ABTS assay, the high temperature was recommended to enhance the chemical kinetics between target compounds and ABTS radical. Moreover, 0.30 mM ABTS concentration and acetonitrile-based mobile phase were suitable to stabilize the ABTS absorbance during the measurement. Preparing ABTS solution with 100 mM PB was suggested to adjust pH during the chemical reaction. These findings could contribute to the development of the utility and enhancement of the value of the on-line HPLC-ABTS assay.

<Chapter 5>

The objective of this study was to obtain beneficial ingredients from CN peels to benefit the food industry and contribute ecologically and economically. The yields of ingredients were 26.2-31.6 mg/g dried material (DM) in pectin, 9.22-11.3 g/100 g fresh material (FM) in insoluble dietary fiber, 5.56-7.15 g/kg FM in peel essence, 23.8-27.0 mg/g DM in naringin, and 8.30-10.2 mg/g DM in neohesperidin. Naringin and neohesperidin were predominant flavonoids in CN peel. Although CN peel pectin had lower viscosity than commercial pectin, it could be applied to smooth texture products. A sensory evaluation by consumer panelists (n=31) and instrumental assays were performed to evaluate aqueous solutions flavored with 0.02 % CN peel essence. The CN-flavored solutions had a preferred smell over commercial citrus-flavored water and were classified into the same group with commercial citrus beverages in the electronic nose assay. The CN-flavored solution had a sourness, bitterness, and orange-like taste, and the overall acceptance of the CN-flavored solution did not significantly differ from commercial flavored water. Given that 1000 tons/year of CN peels are obtained, it provides 5.56-7.15 tons/year of peel essence, equivalent to 55.6-71.5 million bottles/year when 500 ml bottle beverages containing 0.02% CN peel essence are produced. CN peels have the potential as additive agents and ingredients for food, pharmaceutical, and cosmetic products.

<Chapter 6>

In this present study, a model case for food processing waste utilization was established using CN. In the utilization flow of CN peel, 5.56-7.15 g of peel essence, 5.05-6.12 g of water-soluble pectin, 4.71-5.22 g of naringin, 1.61-1.96 g of neohesperidin, and 92.2-113 g of insoluble dietary fiber were obtained from 1 kg of CN peel. Since the extraction methods used in this study were simple and available for food use, they can be employed as a CN processing waste utilization way in the food industry field. The model case of CN processing waste utilization can also be applied to other plant food processing wastes. These may contribute to solving and reducing the problem of food processing waste discharged at 8.36 million tons annually in Japan. Another outcome of this study was the possibility of improving analytical methods. In Chapter 4, consideration of experimental conditions to improve the reactivity of the on-line HPLC-ABTS assay was performed. The optimized method found in this study could contribute to developing the utility and enhancement of the value of the on-line HPLC-ABTS assay in the academic field.