Valorization of the Sea Buckthorn (Hippophae rhamnoides) Pomace for Potential Food Application

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Abstract of thesis

Sea buckthorn (*Hippophae rhamnoides*) berries have attracted growing interest in recent years, owing to their valuable chemical composition, biological properties and people's preference for natural bioactive compounds. The berries are rich in fat-soluble compounds such as β-carotene, lycopene, tocopherol and phytosterols. These compounds have numerous biological properties, including antioxidant and antidiabetic activities, and the ability to reduce the risks of cardiovascular diseases. Processing of sea buckthorn berries generates large amounts of by-products that are not efficiently valorized. The pomace obtained after their juice extraction is considered as by-product and is primarily used as animal feed. This by-product is rich in various bioactive compounds, such as phytosterols and carotenoids, having potential to be used for the development of natural nutritional food and supplements. The conventional extraction method for bioactive compounds commonly uses organic solvent. Despite their efficiency, the organic solvent extraction methods require large quantities of solvents, which are costly, environmentally hazardous, and require expensive disposal procedures. In addition, oil-assisted extraction has limited efficiency because of the low diffusion properties of vegetable oils. Thus, inexpensive and efficient alternate extraction systems are needed to eliminate problems mentioned in the former processes.

In this study, oil-in-water (O/W) emulsion-assisted extraction and oil extraction were evaluated as an alternative to organic solvent. The approach with O/W emulsions is considered green and novel technique for extraction system because of their capability to incorporate different bioactive due to the presence of both lipophilic and hydrophilic domains. This approach also does not involve any hazardous substances during the extraction process. Previous research has been carried out using microemulsion-assisted extraction of bioactives using various synthetic emulsifiers; however, no reports are available on emulsion-assisted extraction system without emulsifier.

The objective of this study was to investigate the efficiency of extraction systems using O/W emulsion as well as various organic solvents and oils on the yields of β-sitosterol and total carotenoids from sea buckthorn pomace. Using obtained optimum extraction condition of time and temperature, the long-term stabilities of β-sitosterol and carotenoids and color change of the extract were also investigated.

The author used dried sea buckthorn pomace, consisting peels and seeds from Mongolia. Organic solvents (ethanol (99.5%), acetone, chloroform, ethyl acetate and *n*-hexane), triacylglycerols (refined soybean oil and rapeseed oil) and medium-chain triglyceride (MCT) oil and emulsifier (Polyoxyethylene 20 sorbitan monolaurate (Tween 20)).

For extraction, the author used 2 g sample of dried pomace was mixed with 20 mL of a given solvent and oil then stirred at 25 °C for 24 h. The suspensions were sonicated for 1 h, and then centrifuged at 9100 *g* for 1 h to remove the undissolved particles. The supernatant was finally filtered (hydrophilic PTFE, size 0.45 μm), and the solvent was evaporated using a rotary evaporator. After evaporation, each residue was used for the analysis of β-sitosterol and carotenoids. The oil extract supernatants were directly used for analysis without evaporation. For the emulsion-assisted extraction, O/W emulsions were prepared by homogenizing 20 mL of the oil phase (refined soybean, rapeseed and MCT oils) with 80 mL of aqueous phase, with and without 1% (w/w) Tween 20. Coarse emulsions were initially prepared using a rotor-stator homogenizer (RSH) at 7000 rpm for 5 min. Fine emulsions were subsequently prepared by high-pressure homogenization (HPH) of the coarse emulsions at 100 MPa for 4 passes. Prepared emulsions (100 mL) were then mixed with 2 g of dried pomace and stirred at 750 rpm at different temperatures (25, 50, 65, 75, and 80 °C), and extraction times (1, 2, 5 and 24 h). The suspensions were sonicated for 1 h at 25 °C, and then centrifuged at 9100 *g* for 1 h to remove the undissolved particles. The supernatant oil phase was used for analyzing β-sitosterol and carotenoids and color change measurement. The β-sitosterol and carotenoids were analyzed by using a UV spectrophotometer at a wavelength of 640 and 450 nm, respectively. The droplet size and size distribution of the prepared emulsions were measured using a laser diffraction particle size analyzer. Total color change was measured by Spectrophotometer CM-5 machine reported as L^* (lightness), a^* (redness) and b^* (yellowness) values. The analysis of variance (ANOVA) was used to compare the extraction yield under different treatment conditions at a 95% confidence level ($p < 0.05$) using SSPS Statistic 8.1 software.

O/W emulsions were found to have much better extraction efficiencies than organic solvents and oils. The author also found that HPH treated emulsions having smaller droplet sizes provided better yields of β-sitosterol (41.9 mg/g dw) and carotenoid (1.42 mg/g dw) than RSH emulsions yields of β-sitosterol (27.9

mg/g dw) and carotenoid (1.17 mg/g dw). The O/W emulsion without emulsifier showed better yields for both β-sitosterol and carotenoids than emulsions with emulsifier.

For organic solvent extraction, their performance was lower than O/W emulsion-assisted extraction, which was speculated that expansion of sea buckhorn cells due to osmotic pressure caused higher extraction of bioactives. The author analyzed using solubility parameter “distance” (Ra) using Hildebrand solubility parameters. The yields of β-sitosterol and carotenoids were plotted against Ra, showing negative correlation was observed (correlation coefficients were around 0.5–0.6), which means that the solvents having close “distance” parameter between the solvents and targeted bioactives would be better.

Among these emulsifier-free systems with HPH treatment, the optimum extraction condition was soybean O/W emulsion use at 65 ℃ for 1 h, giving 32.0 mg/(g dry pomace) and 1.44 mg/(g dry pomace) of β-sitosterol and carotenoids, respectively. The stability of the extracted bioactives was also investigated. The bioactives were relatively stable at temperatures of 5 and 25 °C, but became unstable at 50 °C. For the emulsifier-free soybean O/W emulsion extract, total color faded to orange to yellow during 28 d of storage at 50 °C. However, total color did not change at 5 ℃ and 25 °C. At 50 °C *L** value increased and *a, *b values decreased during storage. The carotenoids degradation rate was lower than β-sitosterol degradation rate. The activation energies for thermal degradation rates of β-sitosterol and carotenoids were estimated to be about 14.51 kJ/mol and 24.3 kJ/mol.

The author concluded that the emulsion-assisted extraction method without emulsifier is a promising technique for the extraction of β-sitosterol and carotenoids that will be subsequently applied in dietary nutritional supplements and fortified food. This developed extraction method can be used for many other bioactives application of dietary from various bioresources.

Abstract of assessment result

【Review】

The fruit pomaces are promising sources of valuable compounds which may be used other applications because of their nutritional properties. The consumers are increasingly aware of diet related health problems; therefore, demanding natural ingredients which are expected to be safe and health-promoting. The solvent extraction is one of the methods to separate bioactive compounds of plant from the inactive components by using solvents. The problem with most commonly used solvents is their negative impacts on health, safety and environment. Recently, there has been an increasing interest in using new eco-friendly extraction system instead of conventional solvents.

The purpose of this study was to investigate the efficiency of extraction systems using various organic solvents, oils and O/W emulsions on the yields of β-sitosterol and carotenoids from sea buckthorn pomace. The results showed that emulsifier-free O/W emulsion-assisted extraction was the best as a green extraction method, compared with solvent extraction systems. The O/W emulsions extract contained higher amount of

β-sitosterol and carotenoids which can be applied in dietary nutritional supplements and fortified food. In addition, the β-sitosterol and carotenoids were also relatively stable at lower and ambient temperatures during 28 d and extract color did not change. However, for successful applications in the future, it would be important to characterize more O/W emulsions extract.

【Result】

The final examination committee conducted a meeting as a final examination on January 17, 2020. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Food Innovation.