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Emulsion-based extraction of β-sitosterol and carotenoids from sea buckthorn (*Hippophae rhamnoides*) pomace

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Abstract

In the present work, we investigated oil-in-water (O/W) emulsion-based extraction of β-sitosterol and carotenoids from sea buckthorn pomace. We compared this new green extraction method with conventional extraction using organic solvents and oils. The objective of the present work was to evaluate the efficiency of these different extraction systems on the yields of bioactive compounds from plant-based materials, and to determine the optimum extraction conditions for maximum extraction yield. Our results indicated that O/W emulsions, prepared without emulsifier using a high-pressure homogeniser, had the highest extraction capability for β-sitosterol and carotenoids, as compared to the other extraction systems. The optimum conditions were 65°C for 1 h extraction, using emulsifier-free soybean O/W emulsions. Under these conditions, the extracted amounts were up to 32.0 mg/g dw (dry weight) for β-sitosterol and 1.44 mg/g dw for total carotenoids. The obtained compounds were relatively stable at 5°C and 25°C for up to 28 days of storage. This emulsion-based extraction method is promising for the extraction of β-sitosterol and carotenoids that can be further applied into dietary nutritional supplements and fortified food.

Introduction

Sea buckthorn (*Hippophae rhamnoides*) berries have attracted 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 (Beveridge *et al.,* 1999). These compounds have numerous biological properties, including antioxidant and antidiabetic activities, and the ability to reduce the risks of cardiovascular diseases (Stobdan *et al.,* 2013). Processing of sea buckthorn berries generates large amounts of by-products that are not efficiently valorised. The pomace obtained after their juice extraction is considered as a waste product and is primarily used as animal feed. This pomace by-product is rich in various bioactive compounds such as phytosterols and carotenoids that have great potential to be used for the development of natural nutritional foods and supplements. The phytosterols (mostly β-sitosterol) have multiple bioactive properties and cholesterol-lowering effects (Jones *et al.,* 1997; Hicks and Moreau, 2001). Particularly, they are used for treating diabetes and bacterial inflammation, and for reducing the risk of heart diseases and cancers. Carotenoids are also well documented for their beneficial biological activities, such as strengthening the immune system, and reducing the risk of cancer and heart diseases (Van Poppel and Goldbohm, 1995; Kritchevsky, 1999).

The conventional method using organic solvent is widely used for the extraction of bioactive compounds (Sabir *et al.,* 2005). However, despite their efficiency in some cases, the organic solvent

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extraction methods require large quantities of solvents, which are costly, environmentally hazardous, and require expensive disposal procedures (Mustafa *et al.,* 2012). Previous studies have reported the extraction of β-sitosterol from sea buckthorn pomace using vegetable oils or supercritical carbon dioxide (Cossuta *et al.,* 2007; Chemat *et al.,* 2012). However, oil-assisted extraction has limited efficiency because of the low diffusion properties of vegetable oils, even at high temperatures, and supercritical fluids are quite costly for production on a large-scale (Goula *et al.,* 2017). Thus, inexpensive and efficient alternate extraction systems are needed to eliminate problems mentioned in the former processes.

In the present work, emulsion-based extraction was evaluated as an alternative to organic solvent and oil extraction. Emulsions are homogeneous mixtures of two or more immiscible liquids, with one of the liquids being dispersed in the other liquid(s) in the form of small spherical droplets (Gadhave and Waghmare, 2014). This approach is considered green and novel technique for extraction system because of their capability to incorporate different bioactives due to the presence of both lipophilic and hydrophilic domains, and does not involve any hazardous substances during the extraction process (Roohinejad *et al.,* 2014). Previous studies have reported the application of microemulsion for the extraction of bioactive compounds from solid plant-based materials such as carrot and tomato pomace (Roohinejad *et al.,* 2014; Amiri-Rigi *et al.,* 2016; Amiri-Rigi and Abbasi, 2019). The extraction of β-carotene using microemulsions was found to be much more efficient than extraction using hexane or glycerol monocaprylocaprate oil (Roohinejad *et al.,* 2014). In previous studies, the high concentrations of synthetic emulsifiers used resulted in the expansion of the cells due to high osmotic pressure and improvement in the overall extraction efficiency (Roohinejad *et al.,* 2014). However, there have been an increase in safety concerns on the use of synthetic emulsifiers for food and cosmetic applications (Flanagan *et al.,* 2006). Therefore, while extensive research has been carried out on microemulsion-based extraction of bioactive compounds using various synthetic emulsifiers, no reports are available on emulsion-based extraction system without emulsifier. This approach is essential to reduce the use of synthetic emulsifier in food and pharmaceutical products, and for increased acceptability by general consumers.

Here, we investigated the efficiency of emulsion-based extraction systems of β-sitosterol and carotenoids from sea buckthorn pomace as compared to organic solvent and oil extraction. We also evaluated the effect of emulsifier addition on the extraction efficiency of bioactive compounds using O/W emulsions. The optimum condition for extraction was determined based on the extracted yields, and the chemical stability of β-sitosterol and carotenoids during long-term storage was also examined.

Materials and methods

Materials

Dried sea buckthorn pomace, consisting peels and seeds, was supplied by Eco-Erdene LLC (Ulaanbaatar, Mongolia). Ethanol (99.5%), acetone, chloroform, ethyl acetate, and hexane were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Triacylglycerols of refined soybean oil and refined rapeseed oil purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and refined medium-chain triglyceride (MCT) oil provided by Taiyo Kagaku Co., Ltd. (Yokkaichi, Japan) were used for oil-based extraction. Polyoxyethylene 20 sorbitan monolaurate (Tween 20) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Water (18 MΩ cm) obtained from an ultrapure water system (Arium® pro, Goettingen, Germany) was used to prepare all the emulsions.

Organic solvent extraction

A 2 g sample of dried pomace was mixed with 20 mL of a given solvent and stirred at 25°C for 24 h. The suspensions were sonicated for 1 h, and then centrifuged at 9,100 *g* for 1 h to remove the undissolved particles. The supernatant was finally filtered (PTFE-0.45 μm), and the solvent was evaporated using a rotary evaporator (Eyela EVP-1100, Tokyo Rikakikai Co., Ltd., Tokyo) at 35°C. Following evaporation, each solid part, known as organic solvent extract residue, was used for the analysis of β-sitosterol and carotenoids.

Oil-based extraction

For oil-based extraction, the solid-to-liquid ratio and extraction parameters were similar to those used in the aforementioned organic solvent extraction. The supernatants were directly used for analysis without evaporation, because of their composition.

O/W emulsion-based extraction

O/W emulsions were prepared by homogenising 20 mL of the oil phase (refined soybean or rapeseed oil) with 80 mL of aqueous phase, containing 1% (w/w) Tween 20, unless otherwise stated.

Coarse emulsions were initially prepared using a rotor-stator homogeniser (Polytron, PT-3000 Kinematica-AG, Littace, Switzerland) at 7,000 rpm for 5 min. Fine emulsions were subsequently prepared by high-pressure homogenisation of the coarse emulsions (Nano Vater, NV200, Yoshida Kikai Co., Ltd., Nagoya, Japan) at 100 MPa for four 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, and 5 h). The suspensions were sonicated for 1 h at 25°C, and then centrifuged at 9,100 *g* for 1 h to remove the undissolved particles.

β-sitosterol and carotenoid analysis

The β-sitosterol content was determined following the method of Daksha *et al.* (2010). Briefly, each organic solvent extract residue (about 0.3 g) was re-dissolved in 10 mL of chloroform, from which, 3 mL of the sample solution were mixed with 2 mL of Liberman-Burchard reagent (0.5 mL of sulphuric acid dissolved in 10 mL of acetic anhydride) and 2 mL of chloroform, and incubated in the dark for 15 min. The absorbance was then measured at 640 nm using a UV spectrophotometer (V-530, Jasco Corporation, Tokyo, Japan). In the case of oil and O/W emulsion extraction, 1 g of the supernatant was mixed with 10 mL of chloroform and analysed using the same method as previously described.

Carotenoids were determined directly at 450 nm using a UV spectrophotometer. For oil and O/W emulsion extract samples, 200 µg of the supernatant was mixed with 5 mL of chloroform, and then measured at the same wavelength as previously described.

Calculation of β-sitosterol and carotenoid contents

The content (%) and extraction yields (mg/g dw) of β-sitosterol and carotenoid from dried sea buckthorn pomace, were determined using Equations 1 and 2, respectively:

Compound content in extract (%) = [Targeted compound weight(g) / Recovered extract weight (g)] × 100 (Eq. 1)

Extraction yield, mg/g dw = β-sitosterol or carotenoids weight in the extract(mg) / Dried pomace weight (g) (Eq. 2)

Measurement of droplet size and size distribution

The droplet size and size distribution of the prepared emulsions were measured using a laser diffraction particle size analyser (LS 13320, Beckman Coulter, Inc., Fullerton, USA). The refractive index used for water was 1.33, and that for soybean and rapeseed oils was 1.47. The average droplet diameter values were reported as volume mean diameter ($d_{4,3}$):

$$d_{4,3} = \sum ni d_i^4 / \sum ni d_i^3 \quad \text{(Eq.3)}$$

where, d_i = diameter, and ni = number of droplets having diameter d_i.

Statistical analysis

All experiments were repeated at least three times per sample. The analysis of variance (ANOVA) was used to compare the extraction yield under different treatment conditions at 95% confidence level ($p < 0.05$) using Statistix 8.1 software (Tallahassee, USA).

Results and discussion

Organic solvent extraction

The β-sitosterol yield and content in extracts, obtained using various organic solvents with different dielectric constants, are shown in Figure 1a. Overall, 50% (v/v) aqueous-ethanol resulted in the lowest extraction yield, whereas extraction with other organic solvents resulted in comparatively similar yields. The yield and content of β-sitosterol were in the range of 5.45 - 8.27 mg/g dw and 3.02% - 6.03% (w/w), respectively. The highest yield of β-sitosterol was obtained using hexane. In Figure 1b, the yield and content of carotenoids are shown to be in the range of 0.69 - 1.12 mg/g dw and 0.46% - 0.75% (w/w), respectively. The highest yield of carotenoids was obtained from acetone and ethyl acetate extraction, whereas chloroform was a less efficient solvent. The lowest yield of carotenoids was obtained for 50% (v/v) aqueous-ethanolic extract.

Our findings are similar to those of a previous study on corn silk waste, wherein hexane was more efficient for β-sitosterol extraction than acetone and ethyl acetate (Zhang *et al.,* 2017). In addition, another study reported that the sterol yield of sugarcane was increased by reducing the organic solvent polarity (Feng *et al.,* 2014), which is in good agreement with our findings. According to Wei *et al.* (2010), the solubility of β-sitosterol (98% purity) was in the following order: *n*-hexane < ethanol < acetone < ethyl acetate. Therefore, the differences observed in β-sitosterol yields across the different organic solvents used in the present work could be due to the difference in solvent polarity, which plays an

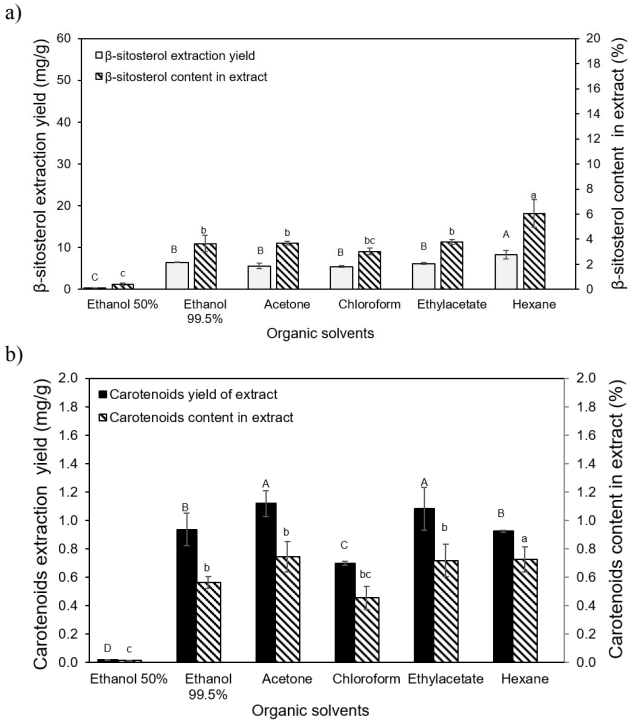

Figure 1. Extraction yield (mg/g) and content (%) of (a) β-sitosterol and (b) carotenoids in sea buckthorn pomace extracts, prepared using different organic solvents.

important role in the extraction of sterols. Ethanol is a polar protic solvent with a dielectric constant of 24, whereas acetone, ethyl acetate, chloroform, and hexane have lower dielectric constants (about 21, 6, 4.81, and 1.88, respectively). Therefore, the use of organic solvents with low dielectric constant is preferable for the extraction of β-sitosterol from sea buckthorn pomace. The results of carotenoid extraction from sea buckthorn pomace by organic solvents is in accordance with previous report, as they found that the efficiency of carotenoid recovery from shrimp waste was in the following order: acetone > ethyl acetate > ethanol > hexane (Sachindra *et al.,* 2006). Furthermore, Strati and Oreopolou (2011) reported that acetone and ethyl acetate were more efficient than other organic solvents for carotenoid extraction from tomato waste. Accordingly, Warkoyo and Saati (2011) observed that acetone was more suitable than ethanol for carotenoid extraction from *Eucheuma cottonii* seaweed. In the present work, acetone and ethyl acetate were more efficient for carotenoids extraction than all the other organic solvents used. The higher yields of β-sitosterol and carotenoids can be obtained by organic solvent extraction than with supercritical fluid extraction, as reported by Cossuta *et al.* (2007) . Their extraction yields were about 2.0 - 4.25 mg/g dw for β-sitosterol and 0.04 - 0.18 mg/g dw for carotenoids, while those in the present work were 5.45 - 8.27 mg/g dw for β-sitosterol and 0.69 - 1.12 mg/g dw for carotenoids.

In general, acetone and ethyl acetate extraction exhibited the highest carotenoids yield among all the organic solvents. This could be due to their polar and water-miscible properties, which may allow for a better extraction efficiency of bioactive compounds from wet plant-based materials. Therefore, in the extraction of wet samples, the use of non-polar solvents may not be advisable, as their penetration through the hydrophilic matrix surrounding the pigment is limited. The pomace used in the present work was obtained in hydrated form and contained mainly polar carotenoids such as zeaxanthin and lutein which may have resulted in better extraction yields with acetone and ethyl acetate.

Oil-based extraction

For many years, the food and cosmetic industries have used oils to extract natural compounds from plant-based materials, as a green and environmentally friendly alternative to organic solvent extraction. Oils also have good dissolving power for hydrophobic bioactive compounds and can prevent their oxidation during extraction, which provides additional advantages for food and pharmaceutical industries. In this section, we used different oils to extract β-sitosterol and carotenoids from sea buckthorn pomace, and compared their efficiency with the other extraction systems.

As shown in Figure 2, the yield of β-sitosterol extracted using different oils was in the range of 7.18 - 12.5 mg/g dw, while that of carotenoids was in the range of 1.03 - 1.15 mg/g dw. Soybean and rapeseed oils resulted in higher yields of β-sitosterol than MCT oil. However, they all provided a similar extraction yield for carotenoids. A previous study reported that soybean oil and MCT oil result in similar extraction yields of carotenoids from shrimp waste (Sachindra and Mahendrakar, 2005). The better extraction yields of β-sitosterol using soybean and rapeseed oils than that of MCT oil can be explained by their long-chain fatty acids composition, which provides increased hydrophobic properties and oxidative stability as compared to the medium chain fatty acids found in MCT (Crozier, 1988; Odle, 1997).

We also observed that oils were able to extract a slightly higher amount of β-sitosterol from sea buckthorn pomace than organic solvents, but the yield of carotenoids was almost similar to the previously obtained results. This is in accordance with previously reported findings, where a good extraction efficiency of carotenoids from pumpkin was

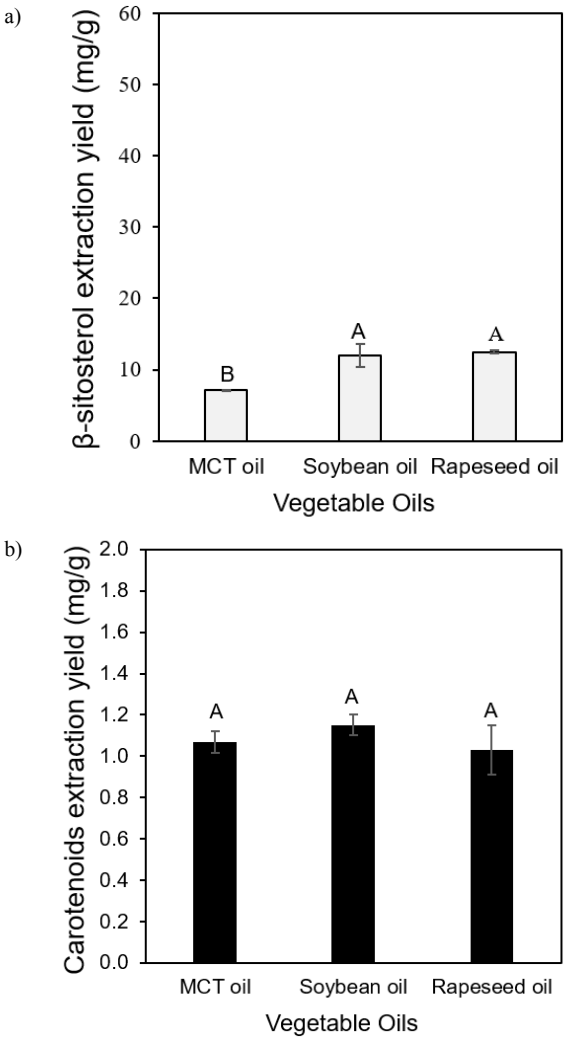

Figure 2. Extraction yield (mg/g) of (a) β-sitosterol and (b) carotenoids in sea buckthorn pomace extracts, prepared using different oils.

observed, using both ethyl acetate and virgin coconut oil (Norshazila *et al.,* 2017). Extraction using oils may be more acceptable than organic solvent extraction, and is considered as a green process due to their environmentally friendly and ability to prevent oxidation. However, this method possesses some limitation, as the oil with high viscosity may hinder oil diffusion through the solid substrate mass and results in low extraction yield even at high temperatures (Goula *et al.,* 2017).

O/W emulsion-based extraction

The increasing attention of emulsion in food formulation mainly originates from its physicochemical properties including low viscosity, thermodynamic stability, and high solubilisation capacity. Emulsions are homogeneous mixtures of two or more immiscible liquids, which can provide particular properties during extraction. Their capability to solubilise lipophilic and hydrophilic substances, lead to an increase of reaction efficacy and selective extraction. In this section, based on our previous result, soybean and rapeseed oils were selected for O/W emulsion-based extraction. As shown in Figure 3, the highest yield of β-sitosterol (41.9 mg/g dw) was obtained using soybean O/W emulsions, whereas rapeseed oil-based emulsions provided the highest yield of carotenoids (1.73 mg/g dw). Interestingly, we obtained about three times higher β-sitosterol yield than with organic solvent and oil extraction. Furthermore, for both bioactive compounds, the addition of emulsifier resulted in lower extraction efficiency by emulsions. The yield of β-sitosterol, for example, decreased from 41.9 mg/g dw to 22.7 mg/g dw, following the addition of Tween 20 during emulsion preparation. The addition of emulsifiers during emulsion formulation may suppress the partitioning of bioactive compounds from the plant matrix to the oil system during extraction, by creating a barrier around the oil droplets. To the best of our knowledge,

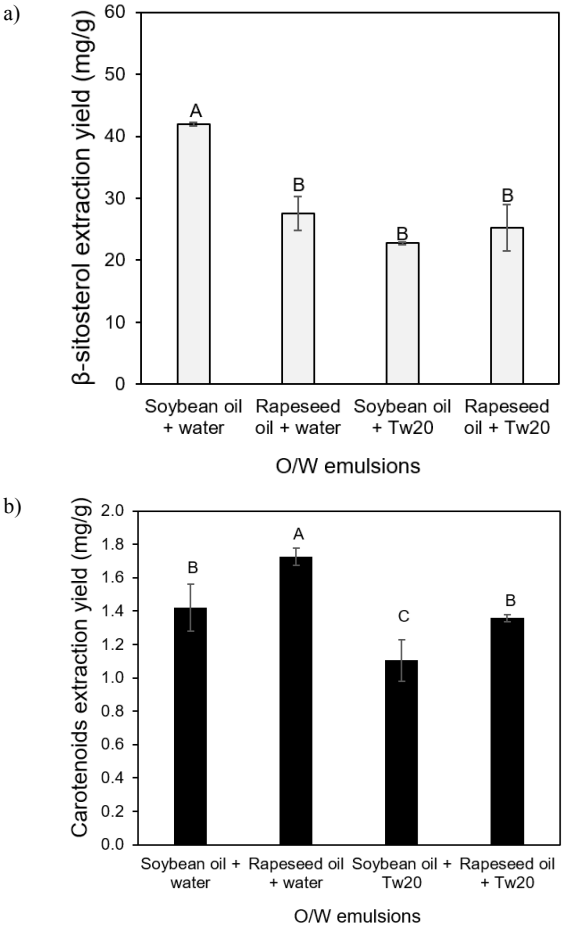

Figure 3. Extraction yield (mg/g) of (a) β-sitosterol and (b) carotenoids in sea buckthorn pomace extracts, prepared using O/W emulsions.

this is the first systematic evaluation of the effect of emulsifier addition on the extraction efficiency of O/W emulsions. Therefore, the addition of emulsifier during emulsion preparation should be further investigated in the future.

Next, we evaluated the effect of emulsion preparation method on the extraction yields of β-sitosterol and carotenoid from sea buckthorn pomace, to evaluate the effect of emulsion properties on extraction efficiency. The emulsions prepared by high-pressure homogenisation (HPH) had a smaller-droplet size ($d_{4,3}$: 2.77 μm) than those prepared by rotor-stator homogenisation (RSH) ($d_{4,3}$: 61.9 μm). The difference in droplet size can be explained by the high energy density input of HPH as compared to that of RSH, which resulted in smaller particle size and more stable emulsions. The HPH emulsions, which have 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).

Overall, the results presented in the present work revealed that O/W emulsions were more efficient than conventional solvent extraction systems for the extraction of bioactive compounds from plant-based materials. Emulsions with smaller droplet size were found to be better than those with large droplets. Roohinejad *et al.* (2014) reported that more carotenoids were obtained using microemulsions than those with hexane or glycerol monocaprylocaprate oil. In their study, the authors used 20% Tween 80 which is considered a high concentration of emulsifier, resulting in the expansion of the cells due to high osmotic pressure and improvement in the overall extraction efficiency (Roohinejad *et al.,* 2014). In the present work, however, emulsions with 1% Tween 20 (lower emulsifier concentration) could extract higher amount of bioactive compounds. Furthermore, this emulsifier-free O/W emulsion was found to give better extraction yields than emulsions with emulsifier.

The mechanism of the emulsion-based extraction can be considered as follows: a) the presence of a high volume of water enhances the swelling of plant cell in the pomace, b) diffusion of oil droplets through the cell membrane, and c) dissolution/solubilisation of the bioactive compounds in the oil. As compared to emulsifier-free emulsion extraction system, it is possible that there was a lower interaction between bioactive compounds and the oil droplets in the emulsion extraction system with emulsifier. This is because the oil droplets were covered by emulsifier and the emulsifier acted as a barrier to prevent the interaction. Our new finding is that emulsifier-free O/W emulsion system was more suitable for the extraction of bioactive compounds, such as β-sitosterol and carotenoids.

Hildebrand solubility is a known theory for solubilisation in extraction systems (Hansen, 2000). The Hildebrand solubility parameter can be used to determine the energy needed to generate space in the molecule to fit other molecules. The solubility parameter "distance" (Ra), between β-sitosterol and carotenoid against organic solvents and oils were calculated based on their respective partial solubility parameter components using Equation 4 (Hansen, 2000):

$$(Ra)^2 = 4(\delta_{D2} - \delta_{D1})^2 + (\delta_{P2} - \delta_{P1})^2 + (\delta_{H2} - \delta_{H1})^2 \quad \text{(Eq.4)}$$

where: δ_{D1}, δ_{D2} = dispersion solubility parameter of material 1 and 2, δ_{P1}, δ_{P2} = polar solubility parameter of material 1 and 2, δ_{H1}, δ_{H2} = hydrogen bonding solubility parameter of material 1 and 2.

For the calculation, cholesterol and β-carotene were used instead of β-sitosterol and carotenoid. The yields of β-sitosterol and carotenoids were plotted against Ra, showing that negative correlation was observed (correlation coefficients were around 0.5 - 0.6). The extraction yields of bioactive compounds increase, if Ra values increase. When compared with the one-phase solvent extraction systems, the two-phase O/W emulsion systems showed significantly higher values, which may suggest that dynamics through the cell walls are also an important factor, such as diffusion and dissolution equilibrium.

Effect of extraction conditions on the yields of β-sitosterol and carotenoid from sea buckthorn pomace using O/W emulsions

In this section, we evaluated the effect of extraction conditions on yields of β-sitosterol and carotenoids from sea buckthorn pomace using emulsifier-free soybean O/W emulsions as a model emulsion-based extraction system. As shown in Figure 4a, the yields of β-sitosterol slightly increased upon increasing the extraction temperature from 25 to 80°C, while the yield of carotenoids increased by increasing the temperature from 25 to 65°C. However, the yield of carotenoids decreased after 75°C (Figure 4b), within a short extraction time of 1 h. The highest yield of β-sitosterol (36.5 mg/g dw) was obtained at 80°C, and that of carotenoids (1.44 mg/g dw) was obtained at 65°C, for an extraction time of 1 h. However, any further increase in the extraction temperature or time reduced the yield of carotenoids.

These findings are in agreement with those of previous studies, which reported that temperatures higher than 65°C resulted in increased β-carotene degradation (Baysal *et al.,* 2000). In addition, previous studies suggested that phytosterol recovery can be significantly improved by increasing the extraction temperature (Feng *et al.,* 2014; Zhang *et al.,* 2017). This can be explained by the breakdown of pomace cell walls at high temperatures, which favours the extraction of bioactive compounds and also the reduction of solvent viscosity, which improves the cell penetration.

Next, we evaluated the effect of extraction time on β-sitosterol and carotenoids recovery at 65°C. As shown in Figures 4c and 4d, the yields of β-sitosterol and carotenoid slightly changed after 1 and 2 h of continuous extraction, showing the high efficiency of emulsion extraction systems on the recovery of bioactive compounds from plant-based materials. However, extending the extraction time up to 5 h resulted in a decrease in the concentration of bioactive compounds, suggesting the occurrence of chemical degradation of the extracted compounds at elevated temperatures (65°C). A previous study reported that the extraction time of 1 h as favourable for maximal extraction of β-carotene (Roohinejad *et al.,* 2014). The extracts obtained using soybean O/W emulsions at 65°C for 1 h were selected to evaluate the long-term chemical stability of β-sitosterol and carotenoids at various temperatures, and the following experiment was carried out under these conditions.

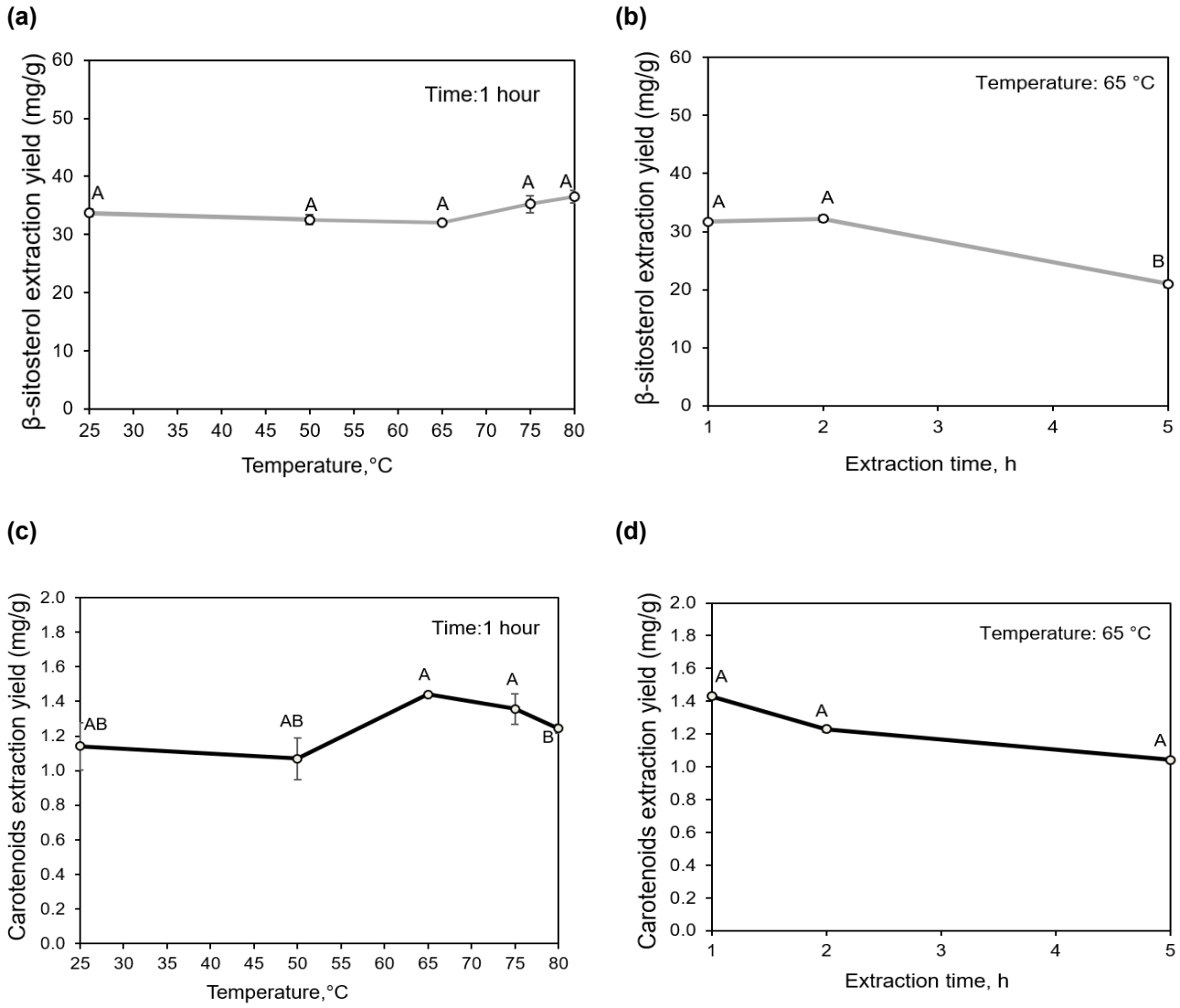

Figure 4. Effect of extraction temperature on (a) β-sitosterol and (b) carotenoid yield; and effect of extraction time on (c) β-sitosterol and (d) carotenoid yield from sea buckthorn pomace using O/W emulsions.

Chemical stability of β-sitosterol and carotenoids in O/W emulsion-based extracts

As shown in Figure 5, the concentrations of β-sitosterol and carotenoids were relatively stable at 5 and 25°C, over the entire period of 28 days of storage. However, they dramatically decreased at 50°C, resulting in an appreciable change of colour. β-sitosterol was more unstable at 50°C than carotenoids due to the rapid degradation of phytosterol at high temperatures. Previous study showed that sterols degrade more rapidly at high temperatures (90°C, 15 min) than at low temperatures (65°C, 24 h) in sterol-enriched milk (Menéndez-Carreño *et al.,* 2008). Moreover, the degradation of sterols in enriched margarine was 1.5-times more rapid at 20°C than at 4°C (Rudzińska *et al.,* 2014). The degradation mechanism of the β-sitosterol is known to be an oxidation process. Our results showed that β-sitosterol was unstable during storage at high temperature and this is probably related to the unsaturated fatty acids in soybean oil, as the radicals from fatty acids and sterols can stimulate oxidative degradation (Dutta, 2004).

Tan *et al.* (2017) reported that the concentration of β-carotene in sunflower oil gradually decreased from 100 to 8%, after 27 days of storage at 37°C. Qian *et al.* (2012) also reported a similar finding on β-carotene degradation in O/W emulsions. However, we found that the degradation of carotenoids in our extracts was slower than that in previous studies. A likely reason for this is the presence of other antioxidant compounds such as tocopherol in our extract, which can prevent oxidation. Overall, it seems that the chemical stability of carotenoids depends strongly on the storage temperature, owing to the presence of multiple double bonds in their structure.

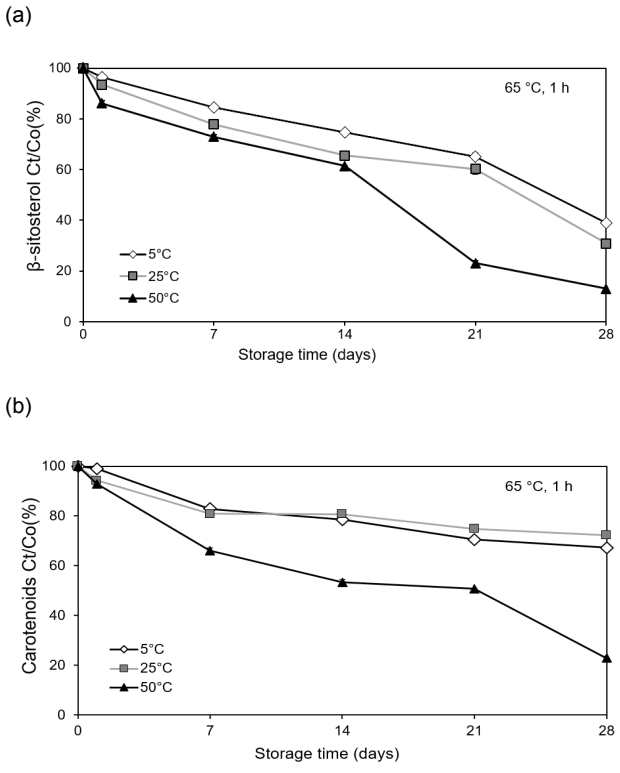

Figure 5. Chemical stability of (a) β-sitosterol and (b) carotenoids in O/W emulsion extracts during storage.

Conclusion

In the present work, O/W emulsion-based extraction system was investigated as a green and efficient extraction method for bioactive compounds. O/W emulsions were found to have much better extraction efficiencies than organic solvents and oils. We also found that HPH treatment, which resulted in smaller droplet sizes, increased the extraction yields, as compared to RSH. The O/W emulsion without emulsifier showed better yields for both β-sitosterol and carotenoids than emulsions with emulsifier. This could be due to the fact that the addition of emulsifier may suppress the partitioning of bioactive compounds from the plant-based material to the oil system. The extracted compounds were relatively stable at lower temperatures (5 and 25°C) during 28 days of storage. This emulsion-based extraction method is a promising technique for the extraction of β-sitosterol and carotenoids that can be subsequently applied in dietary nutritional supplements and fortified food.

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