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Antioxidant, ACE inhibitory, and acetylcholinesterase inhibitory activities of subcritical water extract of blue mussel

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Abstract Subcritical water (SCW) extract of blue mussel was prepared at 100, 200, and 300 °C for 10, 30, and 60 min, respectively, and its effect on the activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, angiotensin-converting enzyme (ACE), and acetylcholinesterase (AChE) was evaluated. We found that DPPH radical scavenging, ACE inhibitory, and AChE inhibitory activities significantly increased with increasing extraction temperature and duration. For example, AChE inhibitory activity of the extract at 300 °C for 60 min increased to 63.1 ± 0.3%, while that at 100 °C for 10 min was 5.6 ± 0.3%. The results suggested that SCW extraction is attractive processing methods for obtaining high valued extract from blue mussel.

Keywords Blue mussel · Subcritical water · ACE · Antioxidant · Acetylcholinesterase

Introduction

Mussels include several families of bivalve molluscs, which inhabit both salt and fresh water, although the term most commonly refers to marine species. The blue mussel (*Mytilus edulis*) is popularly cultivated in South Korea, with approximately 55,000 tons produced in 904 ha of coastal fisheries in 2016 [1]. Blue mussels contain 10.3 g of protein, 5.1 g of carbohydrate, and 1.1 g of lipid per 100 g of edible part [2]. Blue mussels are also rich in polyunsaturated fatty acids. In addition to their nutritional components, several physiological activities, including antimicrobial, antioxidant, and anti-inflammatory activities have been reported [3]. As such, blue mussel is considered a very attractive food material in Korea and other countries, and its consumption has increased. Most blue mussels are distributed in their raw form after harvest, which restricts their usage and application. Therefore, new processing techniques for blue mussel are needed.

Subcritical water (SCW) extraction is an environmentally friendly extraction method which uses water at high temperatures and pressures (100–374 °C, 0.1–21.7 MPa). Water used in SCW is also called superheated water or pressurized hot water. It becomes less polar due to the breakage of hydrogen bonds and behaves like an organic solvent with good solubility for organic compounds. Furthermore, during SCW extraction, organic bonds, such as ester, peptide, and ether bonds, are occasionally hydrolyzed. For these reasons, SCW extraction is an attractive method for the extraction of valuable materials from many food sources, including watermelon [4], maitake [5], and fish meat [6].

In the present study, SCW extract from blue mussels was prepared at 100, 200, and 300 °C for 10, 30, or 60 min, respectively, and the antioxidant, angiotensin-converting enzyme (ACE) inhibitory, and acetylcholinesterase (AChE) inhibitory activities of the extract were then evaluated.

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Materials and methods

Reagents

Rabbit lung acetone powder, hippuryl-L-histidyl-L-leucine (HHL), NAD^+, acetylthiocholine esterase, acetylthiocholine iodide, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and L-tyrosine were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Stainless steel tube (12.7 mm O.D. × 1.2 mm wall × 2 m) and cap for SCW extraction were purchased from Swagelok Co. (Solon, OH, USA). The deionized water used in this study was prepared with a Ultra Pure Water System (RoMax Co., Oxon, UK) with a resistivity of 10–18.3 MΩ cm.

Preparation of SCW extract from blue mussels

The blue mussels used in this study were purchased from a local market in Changwon, Korea in March 2016. They were washed with deionized water and dried in a freeze dryer (Ilshin Lab., Seoul, Korea). A grinder (HBL-3500S, Samyang Electronic Co., Gimpo, Korea) was used to grind the dried blue mussels and the powder was then passed through a 25 mesh sieve (Chunggyesanggongsa, Seoul, Korea). Blue mussel powder (0.1 g) was inserted into a stainless steel tube (14 × 1 cm^2) with 10 mL of deionized water, capped, and placed in a furnace (Daeil Engineering, Seoul, Korea) at 100, 200, or 300 °C for 10, 30, or 60 min, respectively. After hydrothermal treatment, the reaction vessel was cooled at 20 ± 2 °C for 30 min. The reactant was filtered through 0.2-μm filter paper (Advantec, Tokyo, Japan), and the filtrate was stored in a deep freezer (Operon Co., Seoul, Korea) at − 70 °C for further experiments.

Concentration of SCW extract from blue mussels

After preparation of three different SCW extracts from blue mussel at each condition, the extracts at each condition were combined and filtrated through Whatman No. 3 micro filter (0.2 μm) twice. The filtrate was lyophilized, then dried materials were weighed. Concentration of the extract was expressed as average value of dried weight in water (mg/mL).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (RSA)

The SCW extract was diluted with two volumes of deionized water and the DPPH RSA of the extract was determined according to the method described by Lee and Lee [7].

ACE inhibitory activity

The ACE inhibitory activity of three-fold diluted blue mussel SCW extract was determined according to the methods described by Kim et al. [8] and Cushman and Cheung [9].

AChE inhibitory activity

The AChE inhibitory activity of three-fold diluted blue mussel SCW extract was evaluated by the method described by Lee and Lee [7].

Statistical analysis

All data are presented as mean ± SD. Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA). Significant differences between groups was assessed with one-way analysis of variance (ANOVAs) followed by Duncan's multiple range tests or Student's t tests. A p value < 0.05 was considered to indicate statistical significance.

Results and discussion

Concentration of blue mussel SCW extract

The concentration of each SCW extract was shown in Table 1. The extraction was significantly affected by SCW condition. The extraction was carried out at 10 mg/mL, and thus almost components of blue mussel were extracted at 200 °C for 60 min (9.9 mg/mL). On the other hand, the concentration did not increase with increasing SCW temperature or time. It might be because SCW could increase extraction of hydrophobic materials and/or could hydrolyze some covalent bonds. Another possibility is that the extracted materials could be coagulated, and thus not be passed through filter during cooling time after SCW extraction. Therefore, further study is needed to understand on how extraction condition affect the concentration of extract.

Table 1 Concentration of subcritical water extract from blue mussel concentration (unit: mg/mL)

Temperature (°C)	Time (min)		
	10	30	60
100	6.4	6.3	5.6
200	6.6	7.4	9.9
300	7.9	6.9	5.2

DPPH RSA of blue mussel SCW extract

After diluting the blue mussel SCW extract with two volumes of distilled water, the DPPH RSA was determined to evaluate antioxidant activity. As shown in Table 2, the conditions of SCW extraction significantly affect the DPPH RSA of the blue mussel extract. The DPPH RSA of the extract increased with increasing temperature and time. At 300 °C, the RSA increased from 23.9 ± 1.2% for 10 min extraction to 67.0 ± 0.2% for 30 min extraction; however, it sharply decreased to 31.1 ± 1.8% after 60 min of extraction. The highest DPPH RSA (74.2 ± 0.6%) was found when the extract was prepared at 200 °C for 60 min, and was almost 4.5 times higher than the RSA of the 100 °C for 60 min extract (16.5 ± 0.6%). Considering the concentrations of extracts (Table 1), DPPH RSA of the extract was not proportionally increased with its concentration. Ascorbic acid, a positive control, showed 88.1 ± 0.3% DPPH RSA at a concentration of 50 μg/mL.

Blue mussels have been reported to possess significant DPPH RSA. Kim et al. [8] found that a hot water extract of blue mussel flesh showed 36.81% DPPH RSA at a concentration of 10 mg/mL. Lee et al. [10] also reported that ethanol and methanol extract from raw or freeze-dried blue mussel powder showed significant DPPH RSA; however, the water extract at 25 °C for 12 h did not show detectable DPPH RSA. These reports suggest that antioxidant materials in blue mussels are difficult to dissolve in cold water and relatively easy to dissolve in organic solvents and hot water. Carr et al. [11] reported several cases in their review, in which the dielectric constant of SCW was tuned to mimic the dissolving power of organic solvents for non-polar compounds, in order to selectively extract a large number of hydrophobic compounds. The dielectric constant of water, one of the indicators of polarity, decreases with increasing temperature (for example, 55.43, 34.75, and 20.26 at 100, 200, and 300 °C, respectively) [12]. On the contrary, Wang et al. [13] purified antioxidant pentapeptides (Tyr-Pro-Pro-Ala-Lys) from the enzymatic hydrolysate of blue mussel proteins, and Cheng et al. [14] extracted antioxidant polysaccharides from blue mussels. Therefore, the increased DPPH RSA of the blue mussel SCW extract might be due to not only its capacity to solubilize hydrophobic compounds, but also its hydrolytic activity. However, extraction at 300 °C for 60 min seems too harsh for the antioxidative compounds in blue mussels to tolerate.

ACE inhibitory activity of blue mussel SCW extract

As shown in Table 2, ACE inhibitory activity was significantly affected by the extraction conditions. At 100 and 200 °C, ACE inhibitory activity increased with increasing extraction time. However, similar to DPPH RSA (Table 2), the ACE inhibitory activity drastically decreased after heating at 300 °C for 60 min. The maximum ACE inhibitory activity (88.1 ± 3.4%) of the blue mussel extract was found after extraction at 200 °C for 60 min. As like as DPPH RSA, ACE inhibitory activity of the extract was not enhanced with its increased concentration. Captopril, a positive control, showed 32.6 ± 0.9% inhibitory activity at 0.2 μg/mL concentrations.

Several groups have reported the antihypertensive activity of blue mussel extracts. Je et al. [15] identified ACE inhibitory peptides from blue mussel fermented sauce, and Dai et al. [16] characterized that enzymatically hydrolyzed peptides from blue mussels showed ACE inhibitory activity. SCW at high temperatures could result in the breakage of some covalent bonds, such as ester and ether bonds, and liberation of free compounds from polymeric or bound forms. SCW could also possibly cleave peptide bonds in proteins, similar to enzymatic hydrolysis. The specific sequence after cleavage would depend on the temperature and the target protein [17]. Heating SCW at 200 °C for 60 min seems to produce conditions suitable for the extraction of ACE inhibitory compounds, including the peptides described above. However, heating at 300 °C for 60 min seems too harsh to sustain ACE inhibitory peptides. The results suggested that suitable hydrothermal treatment could increase the ACE inhibitory activity of blue mussel extracts.

Inhibitory activity of blue mussel SCW extract on AChE

AChE inhibitors increase the level of acetylcholine, a neurotransmitter, by preventing the breakdown of acetylcholine, and are suggested as highly promising therapeutic agents for patients with Alzheimer's disease [18]. The AChE inhibitory activity of the blue mussel SCW extract is shown in Table 2. It is interesting that, unlike DPPH RSA and ACE inhibitory activity, AChE inhibitory activity increased with temperature and time, even under the 300 °C for 60 min extraction conditions. When compared to the AChE inhibitory activity of the 100 °C for 10 min extraction (5.6 ± 0.3%), the extract which was heated at 300 °C for 60 min showed an increase of more than 11-fold (63.1 ± 0.3%). However, it was not proportionally increased with the increased concentration of extract. Eserine, a positive control, showed 62.8 ± 0.1% AChE inhibitory activity at a concentration of 0.5 μg/mL.

There are two possible explanations for the AChE inhibitory activity of blue mussel SCW extract. First, SCW could extract natural AChE inhibition compounds present in blue mussels. Blue mussels have been reported to have antioxidant carbohydrates which might show AChE

Table 2 DPPH radical scavenging, ACE inhibitory, and acetylcholinesterase inhibitory activities of subcritical water extract from blue mussel (unit: %)

Temperature (°C)	DPPH radical scavenging activity			ACE inhibitory activity			Acetylcholinesterase inhibitory activity		
	Time (min)			Time (min)			Time (min)		
	10	30	60	10	30	60	10	30	60
100	26.6 ± 0.8^{ax}	19.2 ± 0.2^{bz}	16.5 ± 0.6^{cz}	15.4 ± 2.7^{ay}	16.6 ± 2.9^{az}	21.7 ± 4.3^{ay}	5.6 ± 0.3^{ax}	8.2 ± 2.7^{az}	7.3 ± 1.4^{az}
200	15.2 ± 1.6^{cy}	31.7 ± 1.2^{by}	74.2 ± 0.6^{ax}	14.4 ± 1.9^{cy}	43.9 ± 2.0^{by}	88.1 ± 3.4^{ax}	0.5 ± 0.5^{cz}	11.5 ± 0.7^{by}	31.2 ± 1.3^{ay}
300	13.3 ± 1.2^{cy}	67.0 ± 0.2^{ax}	31.1 ± 1.8^{by}	29.7 ± 2.8^{bx}	52.4 ± 0.6^{ax}	14.9 ± 2.0^{cz}	2.4 ± 0.9^{cy}	60.1 ± 0.4^{bx}	63.1 ± 0.3^{ax}

Data represents the mean ± SD values (n = 3) obtained from three individual experiments

$^{a-c}$Different letters within a row indicate significant differences at $p < 0.05$ by Duncan's multiple range test

$^{x-z}$Different letters within a column indicate significant differences at $p < 0.05$ by Duncan's multiple range test

inhibitory activity [19]. The second explanation is the contamination of the blue mussels by artificial pesticides. Among well-known AChE inhibitors, organophosphates and carbamates are used as pesticides. There are several reports on blue mussel contamination by organophosphates and carbamates [20, 21], because blue mussels ingest and filter large amounts of seawater, which can result in the accumulation of pesticides in the body. Because of this, blue mussels are sometimes used as indicator organisms for marine contamination by pesticides. At this time, it is difficult to know whether the AChE inhibitory activity of the blue mussel SCW extract originated from natural bioactive compounds or contaminating pesticides. Further study is needed to identify which compounds are involved in the AChE inhibitory activity of blue mussels, and the kinetics of enzyme inhibition.

Many bioactive compounds and physiological activities have been reported for blue mussels [3]. Though specific compounds were not identified, our results suggested that some of the physiological activities of blue mussel extracts could be significantly increased by SCW extraction. In this study, SCW improved physiological activities of blue mussel extract, however, optimum SCW condition was different with each specific physiological activity, suggesting that compound(s) required for each activity might be different. Our results might contribute to the production of high valued products with addition of blue mussel extracts.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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