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Production of phytotherapeutics from broccoli juice by integrated membrane processes

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ABSTRACT

An integrated membrane process for the recovery and concentration of phytotherapeutics in broccoli juice was investigated to develop a natural product that could be of interest for food and/or pharmaceutical industry. Following a pretreatment step to remove suspended solids, the juice was ultrafiltered through a 50 kDa MWCO polyethersulfone membrane. The permeate stream was further concentrated by osmotic distillation up to 42.6 °Brix. During ultrafiltration, a complete recovery of sulforaphane, malic acid and citric acid was achieved, where total phenolic content and ascorbic acid were recovered at a ratio of 94.5 and 92.4%, respectively. All the bioactive compounds identified in broccoli juice was well preserved during subsequent 6-fold concentration by osmotic distillation. No significant difference was observed in total antioxidant activity of the juice throughout the operation. The proposed integrated membrane process has shown promising potential to produce a natural concentrate enriched in phytotherapeutics of the broccoli juice.

1. Introduction

Over the past few years, a growing number of epidemiological studies have documented a strong correlation between consumption of *Brassicaceae* vegetables and prevention of the risk of several chronic diseases including various cancer types (Kim et al., 2016; Xu et al., 2016). These effects have been attributed to health promoting bioactive compounds such as glucosinolates, isothiocyanates, phenolic compounds and ascorbic acid (Sánchez-Vega, Elez-Martínez, & Martín-Belloso, 2015). Glucosinolates, a class of sulfur-rich secondary metabolites derived from amino acids, play the major role in the pharmaceutical action of *Brassicaceae* vegetables. These compounds are biologically inert glucosides and must be hydrolyzed by the action of enzyme myrosinase to become biologically active molecules (Radoševic et al., 2017). Since myrosinase spatially separated from glucosinolates in plant tissue, hydrolysis only occurs upon tissue disruption. The breakdown products of glucosinolates possess strong biological activity, among which isothiocyanates have been well documented for their therapeutic, antioxidant and antimicrobial properties. Sulforaphane, derived from the breakdown of glucoraphanin, is by far the most extensively studied isothiocyanate due to its unusually high potency to induce phase II detoxification enzymes and to inhibit phase I enzymes that activate carcinogens (Fahey & Talalay, 1999). Broccoli is known as the best source of sulforaphane so far (Herr & Büchler, 2010). Although therapeutical effective doses of sulforaphane have not yet been determined in clinical studies, mice studies revealed that typical sulforaphane concentration which exhibited cancer preventive effect can be achieved by consumption of six uncooked whole broccoli heads on daily basis, which is hard to realize (Herr & Büchler, 2010; Kallifatidis et al., 2009). Apart from glucosinolates, broccoli contains other health promoting secondary metabolites such as phenolic compounds, which have been extensively studied for their cancer-chemopreventive potential. Broccoli exhibit the highest total phenolic content (TPC) among *Brassicaceae* family (Zhang & Hamauzu, 2004). It is the most important source of flavonols, which occur in the plants as complex conjugates with the main aglycones quercetin and kaempferol. Broccoli is also a rich source of ascorbic acid, recognized as an important antioxidant that plays a crucial role in cellular protection against oxidative stress. As humans are unable to synthesize ascorbic acid, it has to be administered on a regular basis. This vitamin has been also reported to enhance myrosinase activity (Burmeister, Cottaz, Rollin, Vasella, & Henrissat, 2000), thus play a role in higher rate of conversion of glucoraphanin to sulforaphane (Shen, Su, Wang, Du, & Wang, 2010).

In the last few years, extraction, isolation and characterization of natural bioactive compounds from plant material have been the core of intense research for utilization of their health promoting properties in naturally produced pharmaceuticals as safer alternatives to some existing synthetic drugs. In this context, broccoli can serve as a potential source for bioactive safe compounds to be utilized as therapeutics in nutraceutical and pharmaceutical industries and as functional

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ingredients in food industry. Organic solvent extraction is the most common technique to extract bioactive compounds (mainly sulforaphane and phenolic compounds) from broccoli (Chen & Chen, 2013). Despite being efficient, this technique usually requires long period of time, large amounts of organic solvent and harsh conditions such as high temperatures, which could lead to degradation of thermolabile compounds. Moreover, they often leave trace amounts of potentially toxic residual solvent in the extract, which restricts their use for human consumption. Alternative green extraction techniques such as ultrasound assisted extraction, pulsed electric field extraction and super critical extraction have also been used for extraction of bioactive compounds from various plant material; however high capital costs are the major drawbacks limiting widespread use of these processes.

Membrane processes have emerged as a leading technology especially in the separation technology field in the last decades. Up to date, pressure-driven membrane operations including microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) have been proposed for clarification, fractionation and concentration of fruit juices and of plant extracts. In particular, MF, UF and NF are commonly applied for clarification and fractionation, where RO is used for concentration of juices or extracts. However, RO generally used as a preconcentration technique allowing to reach final concentrations of about 25–30 °Brix due to high osmotic pressure limitations, which is quite below the value of 60–65 °Brix achieved by thermal evaporation. Alternatively, osmotic distillation (OD), a non-pressure driven membrane process, can be utilized to remove water selectively from aqueous solutions under atmospheric pressure and at room temperature. In particular, OD is an isothermal concentration driven process, in which vapor transport occurs through a non-wetted porous hydrophobic membrane. Since the driving force is not a hydraulic pressure difference, very high concentrations compared to RO can be achieved by OD process (Onsekizoglu Bagci, 2015). Literature data on potential use of integrated membrane processes (IMP) for recovery and concentration of bioactive compounds from highly valued vegetables is still scarce (Jiao, Cassano, & Drioli, 2004). To our knowledge, there is no published paper in the scientific literature on treatment of broccoli juice by membrane processes. In the view of the health promoting effects of the juice, this study aimed to recover and concentrate bioactive compounds from broccoli juice by IMP, thereby to produce high value-added therapeutic concentrate that can be used in functional food formulations and pharmaceutical products.

2. Material and methods

2.1. Materials

Sulforaphane, Trolox, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), phenolic standards (gallic acid, quercetin, myricetin, luteolin, isorhamnetin, kaempferol, sinapic acid, chlorogenic acid and rutin), organic acid standards (fumaric acid, isocitric acid, malic acid, maleic acid, quinic acid, oxalic acid, succinic acid, tartaric acid and citric acid) were purchased from Sigma (St. Louis, MO, USA). Folin-Ciocalteu phenol reagent, methanol and potassium persulphate were analytical grade. All solvents were HPLC grade.

2.2. Production of raw broccoli juice

Broccoli (*B. oleracea* var. *italica*) was purchased from local farmers in Karaagac region of Edirne province of Turkey. Broccoli juice was prepared by squeezing broccoli with commercial 700 W juice extractor (Philips-HR1861, China) with a yield of 35%. The extracted juice was centrifuged at 8832*g* for 10 min at 25 °C to reduce fouling in UF process. Total soluble solid content (TSS) of broccoli juice remained unchanged after centrifugation. A general scheme of processing of broccoli juice is given Fig. S1.

2.3. Ultrafiltration system and procedures

The centrifuged broccoli juice sample was pretreated by using 100 kDa PES membrane in order to remove suspended solids and macromolecules. The pretreated juice was then ultrafiltered through 50 kDa PES membrane. UF of broccoli juice was performed with a laboratory scale Vivaflow 200 UF unit (Sartorius AG, Gottingen, Germany). Technical properties of Vivaflow 200 UF unit is given Table S1. UF was performed according to the batch concentration mode, in which permeate was continuously collected and the retentate stream were recirculated back to the feed tank up to a weight reduction factor (WRF, defined as the ratio between the initial feed weight and final retentate weight) of about 7. The schematic view of the experimental set-up is shown in Fig. S2. The UF system was operated at a feed flow rate of 15 L/h at a temperature of 25 °C. Permeate flux (J_p) was calculated from the mass of collected permeate measured at regular time intervals by using a digital balance (Ohaus Explorer, Nänikon, Switzerland).

2.4. Measurement of hydraulic permeability and cleaning procedure

The hydraulic permeability of the membrane was determined by the slope of the straight lines obtained plotting the pure water flux values versus applied transmembrane pressure (TMP) (0–1.5 bar). The hydraulic permeability obtained for a new clean membrane was indicated as L_p^0. The hydraulic permeability measured after the treatment with broccoli juice is referred to as L_p^1. After the filtration of the broccoli juice, the distilled water was flushed through the membrane surface at 20 L/h and at a TMP of 0.3 bar for 30 min in order to remove the reversible cake layer and the hydraulic permeability referred as L_p^2 was determined. High flow rate and low TMP was used to avoid pore blocking during the water flushing. After removing the cake layer, a chemical cleaning process was performed by flushing 0.5 N NaOH solution at 25 °C for 60 min at 0.3 bar. The membrane was then rinsed with distilled water for 30 min. The hydraulic permeability measured afterwards was L_p^3 (Cassano, Conidi, & Drioli, 2011).

2.5. Osmotic distillation process

The ultrafiltered broccoli juice was submitted to OD process using a capillary membrane module (MD 020 CP 2 N, Microdyn, Germany) (Table S2). In OD process, the ultrafiltered juice was pumped in the shell side and calcium chloride dihydrate at 65% (w/w) used as stripping solution was pumped in the tube side of the membrane in a counter-current mode by using peristaltic pumps (Masterflex, L/S, USA) at flow rate of 30 L/h on both sides. A schematic view of the experimental set-up is presented in Fig. S3. The initial weight of the stripping solution was eight times higher compared to that of the juice in order to prevent a significant dilution with consequent decreasing of the driving force during the process. The temperature of the juice and the stripping solution was kept constant at 25 ± 1 °C using two heat exchangers (WiseCircu, Daihan, Korea). The temperatures were controlled at the inlets and at the outlets of the membrane module using type J thermocouples. A digital refractometer was used to determine concentrations of broccoli juice and stripping solution (AtagoPAL-3, Tokyo, Japan). The evaporation flux was determined with a digital balance (Ohaus Explorer, Nänikon, Switzerland) by measuring the increase in weight of the stripping solution. After each experimental run, the membrane module was cleaned by a five step cleaning procedure. Firstly, both sides of the membrane were rinsed with deionized water. Then, 1% (w/w) NaOH solution was circulated for 1 h at 30 °C. After a short rinsing with deionized water, a 1% (w/w) citric acid solution at 30 °C was circulated for 1 h. Finally, the circuit was rinsed with deionized water.