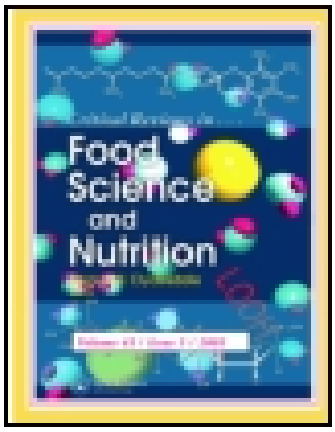

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ABSTRACT

Antioxidants molecules have a great interest for bio-food and nutraceutical industries since they play a vital role for their capacity to reduce oxidative processes. Consequently, these molecules, generally present in complex matrices, have to be fractionated and purified to characterize them and to test their antioxidant activity. However, as natural or synthetics antioxidant molecules differ in terms of structural composition and physico-chemical properties, appropriate separation technologies must be selected. Different fractionation technologies are available but the most commonly used are filtration processes. Indeed, these technologies allow fractionation according to molecular size (pressure-driven processes), charge or both size and charge (electrically-driven

processes). In this context, and after summarizing the reaction mechanisms of the different classes and nature of antioxidants as well as membrane fractionation technologies, this manuscript presents the specific applications of these membranes processes for the recovery of antioxidant molecules.

Keywords: radical-scavenging molecules, fractionation, pressure-driven processes, electrically-driven membrane technologies.

INTRODUCTION

Antioxidants are natural or synthesized molecules present in food according, among others, to the species, the weather and the agricultural practices. They are naturally present at very variable concentrations in fruits and vegetables. Antioxidants play a vital role in both food and chemical systems as well as in the human body to reduce oxidative processes (Van den Ende et al., 2011, Roblet et al., 2012, Rozoy et al., 2012). However, for natural antioxidants, since these molecules are generally present in complex matrices, their separation and purification are required. Indeed, they must be separated from the feedstock in order to increase their purity and antioxidant capacity.

Membrane processes are the main processes used at large scale for the separation of antioxidant molecules. Membranes are selective barriers that allow for the transmission of certain feed components while retaining other components. Membrane technology can be used to concentrate and/or selectively fractionate bioactive compounds with antioxidant activity from aqueous and alcoholic processing streams of products, by-products and wastes from agro-food industry (Díaz-Reinoso et al., 2011). When compared with other concentration methods (evaporation, spray-drying…), the product is not subjected during membrane processes to high temperatures and there is no change in the physical state of the solvent, meaning that the functional properties of the compounds of interest are preserved and the process as a whole is energy saving (Negrão Murakami et al., 2011).

This review article summarizes the reaction mechanisms of the different classes and nature of antioxidants, the membrane separation technologies available and presents the specific applications of these membranes technologies or membrane strategies for the recovery of these molecules of interest.

DEFINITION OF AN ANTIOXIDANT

An antioxidant is a chemical compound or substance that inhibits or retards the oxidation of other molecules (fat, oil, food, …) (Focke et al., 2012, Roblet , et al, 2012, Rozoy , et al, 2012). These molecules are effective at low concentration, and can act as pro-oxidant when their concentration is increased. Oxidation is a chemical reaction where electrons or hydrogens are transferred from a molecule to another molecule. Oxidation is a loss of one or more electrons or protons.

These molecules can act as antioxidant agents according to three different ways:

1) Stop the propagation reaction by exchanging one or more protons with a free radical or a free peroxide radical (phenolic agent)

2) decrease or block the formation of free radicals by complexing metal (chelating agent)

3) decrease the concentration of reactive oxygen or oxygen species (ROS) (oxygen or ROS scavenging agent)

There are two main groups of antioxidants: the primary and secondary antioxidants. The phenolic agents are also called primary antioxidants, while chelating and oxygen/ROS scavenging agents are called secondary antioxidants or synergistic antioxidants. The mechanisms of action are different according to their chemical structure or composition.

ANTIOXIDANTS AND REACTION MECHANISMS

Primary or phenolic antioxidants

Reaction mechanism

The phenolic antioxidants act mainly by preventing the formation of free radicals implied in the process of auto-oxidation. Oxidation reactions can produce free radicals which can start chain reactions. When the chain reaction occurs in food, petroleum products or in a cell, it can cause damage to the products or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit further oxidation reactions (Shipp and Abdel-Aal 2010, Quideau et al., 2011, Van den Ende , et al, 2011). Antioxidant can stop chain reaction by being oxidized themselves. The main role of phenolic antioxidant is to transfer a proton to free radicals, which are very reactive compounds (Fig. 1).

The general reactions can be:

$$AH + ROO\bullet \rightarrow A\bullet + ROOH$$

or $$AH + R\bullet \rightarrow A\bullet + RH$$

or $$AH + RO\bullet \rightarrow A\bullet + ROH$$

where AH is the antioxidant, and **A•** the radical formed. **A•** is relatively stable and do not further react.

Different phenolic antioxidants

The primary or phenolic antioxidants generally used are synthetic antioxidants (BHT, BHA, TBHQ or gallates), but natural antioxidants such as herbal extracts like rosemary and sage, as well as tea extracts have already been commercialized as alternatives to synthetic antioxidants (Shahidi 2000).

Synthetic phenolic antioxidants

BHA, BHT and TBHQ

Butylated hydroxyanisol or BHA (Fig. 2), is composed of two isomeric compounds (forms A and B): the A form is dominating in a proportion of 90% or more. BHT, or Butylated hydroxytoluene, possesses a structure very close to the ones of the BHA isomers (Fig. 2). TBHQ or tertiary butyl hydroquinone (TBHQ) is the more recent antioxidant developed for food applications. It is limited to oil, fat and shortenings.

Gallates

The most common gallates are propyl, octyl and dodecyl gallates. Gallates possess in their structures three hydroxyl groups comparatively to only one for BHA/ BHT and two for TBHQ.

Natural phenolic antioxidants

Tocopherols

Tocopherols are among the main natural phenolic antioxidants. These compounds are naturally present in vegetal oils (Baldioli et al., 1996). There are 4 main types of tocopherols, the α-, β-, γ and δ-tocopherols. The difference between the forms is due to the position of the substituted methyl group (CH_3) (Fig. 3). The α-form is corresponding to Vitamin E. The δ-form is more antioxidant than the other ones (δ > γ > β > α).

Polyphenols

Polyphenols (polymeric phenols) form an important group with more than 10000 individual compounds currently characterised from plants (Quideau , et al, 2011). These compounds have in common one characteristic, the presence of at least one aryl ring on which a hydroxyl group is fixed (Haslam et al., 1988, Bravo 1998). Amongst phenolic compounds, the majority of polyphenols found in food are flavonoids (Fig. 4). Flavonoids can be divided into six classes: flavones, flavanones, isoflavones, flavonols, flavanols and anthocyanins (Wang et al., 2000). Anthocyanins are the most studied polyphenols (Shipp and Abdel-Aal 2010). Anthocyanins are derivatives of anthocyanidins, which include pendant sugars (Fig. 5). The anthocyanins, anthocyanidins with sugar group(s), are mostly 3-glucosides of the anthocyanidins.

Many polyphenols are strong radical scavengers with health promoting effects. They are subjects to an increasing interest due to their potential benefits on human health. Their roles as natural antioxidants could find potential applications for the prevention or treatment of cancer as well as inflammatory, cardiovascular and neurodegenerative diseases. Numerous scientific works are currently under way to demonstrate their real effects on human health.

Protein and peptides

Proteins and protein hydrolysates derived from sources like milk, soy, egg, and fish have also been shown to exhibit antioxidant activity in various muscle foods (Peña-Ramos and Xiong

2003, Sakanaka and Tachibana 2006). The antioxidant activities of all these molecules would be in relation to their composition in amino-acids residues. Well known amino acids residues presenting antioxidant activities are aromatic residues such as tryptophan, tyrosine and phenylalanine and the nucleophilic sulfur-containing amino acids cystein and Methionine. However, free amino acids are not generally found to be effective as antioxidants in food and biological systems (Samaranayaka and Li-Chan 2011).

Several studies reported that the antioxidative activity of protein hydrolysates and isolated peptides were in some cases, similar or higher in activity to that of commonly used synthetic antioxidants such as BHA and BHT (Rajapakse et al., 2005, Je et al., 2007, Samaranayaka and Li-Chan 2008, Samaranayaka and Li-Chan 2011).

Synergistic antioxidants

Synergistic antioxidants are of different natures and possess low or no direct antioxidant properties. However, when these compounds are used in combination with phenolic antioxidants, they have the property to increase considerably the efficiency of phenolic compounds, due to the synergistic effects they procure. These synergistic effects are 1) regeneration of phenolic antioxidants, 2) prevention of the decomposition of peroxides following antioxidant oxidation, 3) inactivation of trace metal catalyzer of oxidation reactions and 4) elimination or decrease of oxygen/ROS concentration.

The synergistic antioxidants can be divided in two main groups, the chelating agents and the oxygen scavenging agents.

Chelating agents

The chelating agents inactivate metal which are susceptible to act as catalyzer of oxidation reactions. The Fenton reaction catalysed by transition metals such as ferrous ion ($Fe^{2+} + H_2O_2 \rightarrow$ •OH + OH^- + Fe^{3+}) is a famous chemical reaction resulting in the production of •OH radicals. In living cells, iron is generally sequestered in redox-inactive complexes to prevent as much as possible the oxidative damage via Fenton chemistry (Prousek 2007).

The chelating agents possess in their chemical structure non-paired electrons, so that they can form stable complexes with metal ions (Fig. 6).

These chelated metal ions are then no more available for catalyzing oxidation reactions and to interact further with phenolic antioxidants. Ethylene diamine tetraacetic acid (EDTA), polyphosphates, tartaric and citric acids are the most common chelating agents. Some peptides from protein hydrolysis also show chelating properties. The most reactive amino acid residue for chelating metal ions is the imidazole-containing amino acid histidin. Carnosine (β-alanyl-L-histidine), anserine (β-alanyl-L-1-methylhistidine), and ophidine (β-alanyl-L-3-methylhistidine) are antioxidative peptides naturally present in muscle tissues (Babizhayev et al., 1994, Chan et al., 1994). Carnosine can act as a free metal ion chelator (Kang et al., 2002).

Oxygen and reactive oxygen species scavenging agents

Reaction mechanisms

The reaction mechanism of oxygen scavengers is to react with free oxygen molecules present in the medium and to make them unavailable for oxidation reactions. This group of antioxidants can eliminate oxygen but also reactive oxygen species (ROS) such as hydroxyl radical (•OH), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$). Concerning ROS, antioxidants act as electron donors, reducing ROS to less harmful molecules ; the oxidised products formed in the process are not very reactive or harmful (Van den Ende , et al, 2011). Among ROS, the •OH is the most reactive (in vivo halflife 10^{-9} s) and dangerous species, immediately attacking virtually any molecule in its neighbourhood (Van den Ende , et al, 2011).

Oxygen/ROS scavengers

Amongst others oxygen or ROS scavengers, we find ascorbic and isoascorbic acids, non-digestible carbohydrates, protein and peptides as well as body enzymes.

Ascorbic and isoascorbic acids

The general oxidation reaction of ascorbic acid and its reaction mechanism with oxygen is presented in Fig. 7.

Sulfites

As ascorbic acid and its derivates, sulfites are good oxygen reductive agents. Generally, sulfites are used as antimicrobial compounds. Indeed, in wine, fruit juices, concentrates and fruit pulps, sulfites are used for their antifungal properties. However, sulfites are also incorporated in food products for their antioxidant and anti-browning activities.

Non digestible carbohydrates

Non digestible carbohydrates (fructans, raffinose family oligosaccharides, arabinoxylans, β-glucans) and their breakdown products (fructosyl oligosaccharides) as well as sugar-sterols and sugar-phenols might act as important ROS scavengers in plants (Nishizawa et al., 2008). The antioxidant capacity of inulin-type fructans can further be increased by grafting extra groups (e.g. phenols) on their structure (Ren et al., 2011).

Proteins and peptides

The higher antioxidative activity of peptides compared to free amino acids is attributed to the unique chemical and physical properties conferred by their amino acid sequences, especially the stability of the resultant peptide radicals that do not initiate or propagate further oxidative reactions (Elias et al., 2008).

Peptides such as glutathione are well-known for their endogenous antioxidative activity (Babizhayev , et al, 1994). Glutathione (γ-Glu-Cys-Gly) is naturally present in muscle tissues (Babizhayev , et al, 1994, Chan , et al, 1994). Glutathione acts as an electron donor to protect cells from free radicals (Bray and Taylor 1994). Its reducing power helps maintain the reduced state of cysteinyl sulfhydryl groups in proteins and thereby reduces disulfide bond formation within cytoplasmic proteins (Bray and Taylor 1994).

Body enzymes

Reactive oxygen species are essential to energy supply, detoxification, chemical signaling and immune function. They are continuously produced in the human body and they are controlled by endogeneous enzymes. These enzymes are superoxide dismutase, catalase and glutathione peroxidase (Dimitrios 2006).

SEPARATION TECHNOLOGIES AVAILABLE

On a large production scale, pressure-driven membrane technologies and electrically-driven membrane technologies have been developed to improve the efficiency of bioactive molecules production.

Pressure-driven processes

The mechanism behind the selectivity is based upon the selective permeability of one or more of the liquid constituents though a membrane; particles or molecules larger than the membrane pores are retained while the smaller ones pass through. The driving force used to achieve the desired hydrodynamic flow though the membrane is a hydrostatic pressure gradient (Rosenberg 1995, Bird 1996). Pressure-driven membrane can be divided according to the pore sizes and the required transmembrane pressure (TMP). Pressure-driven membrane processes include microfiltration (MF, 0.1-5 µm, 1-10 bar), ultrafiltration (UF, 0.5-100 kDa (1nm – 100 nm), 1-10 bar), nanofiltration (NF, 0.1-0.5 kDa (0.5-10 nm), 10-30 bar) and reverse osmosis (RO, < 0.5 nm, 35-100 bar).

Ultrafiltration (UF) and nanofiltration (NF) are often used in the food industry to isolate antioxidant molecules such as specific peptides or peptide fractions (Pouliot et al., 2000, Tessier et al., 2006, Tessier et al., 2006, Vandanjon et al., 2009, Bazinet and Firdaous 2012) and phenolic compounds (Nawaz et al., 2006, Kalbasi and Cisneros-Zevallos 2007, Mello et al., 2010, Tylkowski et al., 2010). Pressure is the main driving force for these processes. The pressure used depends on the pore size of the filtration membrane and has to be adjusted as a function of the concentration rate desired. The solution properties, charges which arise the repulsions and attractions of ions and the type of membrane used affect the behaviour and the efficiency of the separation method (Martin-Orue et al., 1998). Working at low concentration is

preferable to avoid the formation of a polarization layer which could affect the membrane selectivity (Shahidi 2004).

Electrically-driven processes

Electrically-driven processes, like electrodialysis (ED) and hybrid processes (ED with bipolar membranes and ED with filtration membranes), uses ion-exchange membranes and/or filtration membrane(s) to separate molecules. The electric field is the main driving force involved in these processes. Molecular transfer is mainly due to the charge of the molecule and the flux depends on the strength of the electric field (Poulin et al., 2007, Doyen et al., 2012).

Conventional electrodialysis (ED)

Under the influence of an electrical field, ionic (electrically-charged) species are transported, from one solution to another, by crossing one or more perm-selective membranes (Bazinet 2005). Four solutions are circulating at the same time into the electrodialyser: the solution to be treated (diluate), the salt solution (concentrate) and the two solutions at the electrodes (electrolytes). Each solution compartment is connected by an external circuit to a separate reservoir to allow recirculation of the solutions during treatments. The electrodes, at the extremities of the system, are connected to a current generator and allow the transfer of the electricity.

Electrodialysis with bipolar membrane (EDBM)

Bipolar membranes (BPM) appeared commercially at the end of the 1980's. Bipolar membranes have the property to dissociate water molecules under an electric field and to generate a flow of protons H^+ at the cationic interface and a flow of hydroxyl ions OH^- at the anion-exchange interface (Pourcelly and Bazinet 2008).

Electrodialysis with filtration membrane (EDFM)

Electrodialysis with filtration membrane, a technique developed recently by Bazinet et al. (2005), has demonstrated very high selectivity for the separation of organic charged biomolecules. In this system, one or more ultrafiltration membrane is stacked as a molecular barrier in a conventional electrodialysis cell. EDUF couples size exclusion capabilities of UF or porous membranes with the charge selectivity of electrodialysis (ED).

This recent technology allows fractionation of molecules as a function of their charges and molecular weights: according to the molecular weight cut-off of the filtration membrane located in the cell, low molecular weight (MW) molecules cross the membrane while higher MW molecules remain in the feed. Furthermore, according to their charge (mainly driven by the pH of the solution) molecules positively charged migrate towards the cathode, while negatively charged ones migrate toward the anode.

APPLICATIONS TO THE SEPARATION OF ANTIOXIDANTS

This next part presents the different applications reported in the literature of pressure-driven and electrically-driven membrane technologies developed for the recovery of natural antioxidants: tocopherols and tocotrienols, phenols, organic acids (ascorbic, isoascorbis, folic and citric acids) and peptides.

Tocopherols and tocotrienols

The crude oil that is extracted from the biological material is a mixture of fatty acids, mono-, di- and triglycerides, phosphatides (gums), sterols and tocopherols, and pigments (Snape and Nakajima 1996). Tocopherols and tocotrienols are natural antioxidants present in the vegetable oils and have a beneficial effect on product quality (Young et al., 1994). The chemical structures of their isomers and physical properties are well documented (Young , et al, 1994). The most important lipophilic phenols present in olive oil are tocopherols, and nearly 95% of the total ones is alpha-tocopherol (Lozano-Sánchez et al., 2010). The tocotrienol contents are generally very low as compared to tocopherols in crude vegetable oils. Hence, the main applications of tocopherols recovery by membrane are from vegetal oil. However, very few works are reported concerning the use of membrane for their recovery.

MF

The use of MF for the recovery of tocopherols and tocotrienols was indirectly performed by de Souza et al. (2008). They investigated the degumming (removal of phospholipids) of crude oil/hexane miscella, using an alumina multichannel ceramic membrane with an average pore diameter of 0.05 μm. They studied the influence of transmembrane pressure (TMP) (0.5 and 1.5 bar), tangential velocity (1.4 and 2.4 m/s) and percentage of corn oil on the miscella (25% and 35% w/w), in terms of the permeate flux and removal of phospholipids. A phospholipid removal of between 65% and 93.5% was achieved, resulting in a minimum phosphorus content in the permeate of 23 mg/kg and a reduction in color and waxes, besides the conservation of tocopherols and tocotrienols in the crude oil. A raised TMP and a greater percentage of oil in the miscella had a positive effect on the retention of phosphorus, while the tangential velocity had a negative influence. Under the best operational conditions used the permeate flux reached 120 $kg/h.m^2$, at 40°C (de Souza , et al, 2008).

NF

The first works aiming the separation of tocopherols were carried out in 1998 by Subramanian et al. (1998a, b). They demonstrated on a screw-pressed groundnut and sunflower oils, that major tocopherols permeated preferentially through a NF membrane compared to triglycerides increasing consequently the relative amount of tocopherol in the permeates. Experiments were conducted using a flat membrane test cell (model C40-B; Nitto Denko Corporation) under nitrogen atmosphere. The pressure, temperature and stirrer spin bar speed were maintained at 2–

5 MPa, 20–50 °C and 400 rpm, respectively (Table 1). The unit was operated in batch mode by charging the cell with 100 g of model oil and the experiment was stopped when permeate collection was approximately 6–10 g. Polymeric composite membrane, NTGS-2200 with silicon as active layer and polyimide as support layers was used in the study (Nitto Denko Corporation, Kusatsu, Japan) (Subramanian et al., 1998). Tocopherols contents in membrane permeates of groundnut and sunflower oils were much higher than the literature values of the respective refined oils. In another study, Subramanian et al. (1998) on membrane processing of solvent extracted soybean and rapeseed oils observed similar behaviour of tocopherols preferential permeation. These experiments were carried-out with a polymeric NF at a constant pressure of 2-3 MPa. In a more recent study on triglycerides–tocopherols model system, these authors confirmed the tocopherols permeation through nonporous membranes (Subramanian et al., 2003). They also observed that an increase in tocopherols concentration increased the feed viscosity, however, the total permeate flux remained practically constant.

Fractionations of tocopherols and tocotrienols are performed according to similar successive steps which differ only by the filtration process used (Fig. 8). Indeed, the raw material used is usually crude oil. Its separation is performed under nitrogen atmosphere in order to avoid oxidation of molecule by oxygen. The fractionation by MF and NF is carried-out under the best operational conditions in terms of pressure, temperature and flow velocity. After the separation step, the composition of fraction recovered in permeate or retentate was determined by HPLC method to evaluate the impact of the filtration process on the concentration of target molecules.

Phenols

Only recently, researches have begun to consider the recovering of phenols, as high value compounds, transforming some food effluents to raw material with high potential economic value (Russo 2007). For example, polyphenolic compounds with relatively low molecular weights have been found to be responsible for physicochemical deterioration of apple juices and concentrates during storage (Lea 1995, Bazinet et al., 2011). Polyphenols represent the third most abundant constituent in grapes and wines after carbohydrates and fruit acids (Singleton 1982). In the food and dairy industries, both NF and UF are largely used to treat effluents. Applications for the fruit juice and wine industries have recently begun growing in importance (Cissé et al., 2011). Very recently electrodialysis with filtration membrane was proposed for the concentration of anthocyanins (Bazinet et al., 2009, Bazinet et al., 2012).

The phenolic-phenolic interactions or interactions with food components in reversible and irreversible ways could have an impact on membrane filtration process and antioxidant activity. Indeed, polyphenols such as tannins may interact together to form large particles (Riou et al., 2002) which have a negative impact during filtration process (Vernhet and Moutounet 2002). Moreover, polyphenols, such as flavonoids or tannins, can combine with proteins to form soluble complexes. The complexes can grow to colloidal size (Siebert et al., 1996) and may be responsible of a decrease of antioxidant activity (Arts et al., 2002) and a fouling layer formation during microfiltration process (Riedl et al., 1998). Finally, interactions between polyphenol and

polysaccharide, such as pectin (Le Bourvellec and Renard 2011) were reported to increase microfiltration membrane fouling (Czekaj et al., 2000).

MF

In olive oil processing, the three phase continuous process used, produces large amounts of vegetation waters (VW). The most important classes of phenols in olives are phenylacids, phenyl-alcohols, flavonoids and secoiridoids (Macheix et al., 1990). Membrane filtration experiments were carried-out in two plants using ceramic or polymeric MF membranes at TMP ranging from 1.5 to 2 bar (Russo 2007). Free polyphenols with low MW detected in VW were only 15% of those detected in MF 0.45 μm permeate (55.38 ppm vs 349.18 ppm). According to Russo (2007) the free polyphenolic yield in the permeate stream was favoured, during MF process, by the rupture of VW organic aggregates and the release of free polyphenols in solution, due to the mechanical stress during MF operation, their release in solution and consequent permeation. The author concluded that the polyphenols recovery yield could be increased, by a prior hydrolysis step to release free polyphenols in solution, working at higher values of volumetric concentration ratio and with a diafiltration step (DF) (Russo 2007).

Microfiltration (membrane pore size of 0.1 and 0.2 μm) of pineapple juice was reported by Laorko et al. (2010). The authors showed that MF did not affect significantly the pH, reducing sugar and acidity of clarified juice whereas the suspended solids and microorganism were completely removed. The highest permeate flux, total phenolic content and antioxidant capacity were obtained with the 0.2 μm membrane. The optimum operating conditions for the

clarification of pineapple juice by MF was a cross-flow velocity of 3.4 m/s and a TMP of 0.7 bar. An average flux of about 37 L/m^2.h was obtained under the optimum conditions using batch concentration mode (Laorko , et al, 2010).

Microfiltration process, with membrane pore sizes of 0.2 and 0.8 μm, was also used to fractionate açaí pulp for the recovery of phenolic compounds (Machado et al., 2012). Moreover, the effect of pectinase enzyme treatment on açaí pulp, before MF, experiments was studied. The author demonstrated that the highest values of permeate flux and accumulated permeate volumes were obtained with the 0.2 μm membrane when pulp was treated with pectinase enzyme. The optimum condition for the pulp separation was a TMP of 100 kPa and a cross-flow velocity of 3.2 m/s (Machado , et al, 2012).

UF

The different sources studied are olive mill wastewater (Russo 2007, El-Abbassi et al., 2012), fruit juices (Borneman et al., 2001, Cassano et al., 2008, Laorko , et al, 2010, Cissé , et al, 2011, Conidi et al., 2011), grape seeds (Nawaz , et al, 2006), winery sludge (Galanakis et al., 2013), flaxseed hull (Loginov et al., 2013) and green tea (Li et al., 2005).

Russo (2007) tested four UF membranes having different molecular weight cut-off (MWCO, 1 kDa, 6 kDa, 20 kDa and 80 kDa), on vegetation waters (VW) from olive oil processing to recover polyphenols. Fractionations were performed at TMP ranging from 1.5 to 4.5 bar depending on the type of membrane (polymeric or ceramic). Russo showed that it is possible to purify by UF the polyphenolic components contained in a MF permeate. However, the author

observed that the UF membrane tested whatever the MWCO showed the same selectivity for the polyphenols and differ only for the rejection values. In particular UF 6 kDa rejected hydrotyrosol at 45% and UF 1 kDa on UF 6 kDa permeate of other 30%. Yield of polyphenols and in particular of hydroxytyrosol could be increased with a diafiltration step (Russo 2007).

The effects of UF membrane property and operating conditions (MWCO of 30 and 100 kDa, TMP of 2 bar) on the permeate flux, membrane fouling and quality of clarified pineapple juice were studied by Laorko et al. (2010). However, the results were less interesting than those obtained for MF treatment (see previous MF results). On kiwifruit, Cassano et al. (2008) developped a natural product which can be used to fortify foods and beverages by using an UF treatment for concentrating polyphenols among other bioactive compounds. The kiwifruit juice was clarified in optimal operating conditions, according to the batch concentration mode, up to a final volume reduction factor (VRF) of 2.76. An optimal TMP value occurred at 0.6–0.65 bar in different conditions of cross flow velocities and the steady-state permeate fluxes increased linearly between 20 and 30 °C. Most polyphenolic compounds of the depectinised kiwifruit juice were recovered in the clarified fraction of the UF process. The losses of phenolic compounds in permeate and retentate streams, respectively of 13.5% and 25.6%, were attributed to the continuous action of polyphenol oxidases (retained in the retentate). The same research team, develop a similar process on bergamot juice (Conidi , et al, 2011). Bergamot is the common name of the fruit Citrus Bergamia Risso, an endemic plant of the Calabrian region in Southern Italy. The juice, considered as a waste of the essential oil production, contains a considerable amount and variety of flavonoids and flavonoid glycosides having important antioxidant

properties. To develop a natural product enriched in polyphenols, Conidi et al. (2011) proposed a process based on the initial clarification of depectinised bergamot juice by ultrafiltration (UF) at TMP of 0.7 bar, devoted to the removal of suspended solids. The clarified juice was then submitted to different UF processes in order to evaluate the effect of the nominal molecular weight cut-off (MWCO) on the rejection of the membranes towards polyphenols. Results obtained with the UF 1000 Da membrane showed that the physico-chemical properties of the clarified juice were preserved during this process. Only a little reduction of total antioxidant activity (TAA) (9.2%) was observed in the permeate, in comparison with the initial feed. HPLC analyses revealed that, although there was a little increase in the concentration of phenolic compounds on the retentate side, the composition of the permeate and retentate fractions were similar. Similar results were obtained by Wei et al. (2008) in the UF of apple juice with a Pellicon-2 regenerated cellulose membrane having the same MWCO. The effect of the pH (2-5) and of the TMP (3-5 bar) on the rejection of this membrane towards polyphenols was also evaluated: an increase in pH from 2.8 to 8.5 increases the rejection from 0.9% to 8.6% (Conidi , et al, 2011).

On roselle (Hibiscus sabdariffa L.) extract, ultrafiltration membranes with eight different MWCOs ranging from 1 to 150 kDa were tested to concentrate the anthocyanins (Cissé , et al, 2011). A pilot system with an effective membrane area of 0.0155 m^2 was used. It was observed that the retention values of total soluble solids, acidity and anthocyanins increased with transmembrane pressure. Total anthocyanin retention ranged from 24% for UP 150 membrane (150-kDa MWCO) at 0.5 MPa to 97% for UP 005 (5-kDa MWCO) at 3.0 MPa. The high retention levels obtained with the UF membranes were surprising since the molecular weight of

the solutes retained are low (around 600 g/mol for anthocyanins). The authors explained that this high retention would not be due to steric restriction, but to membrane/solutes interactions. In addition, the authors demonstrated that retention of anthocyanins decreased in logarithmic proportion as the nominal MWCO increases, allowing prediction of retention values according to a membrane's MCWO. Hence, they calculated that for a membrane with a 5-kDa MWCO, retention of total anthocyanins varied between 93% and 97% when the TMP increased from 1 to 3 MPa. For a membrane with a 150-kDa MWCO, the retention of anthocyanins ranged between 28% and 62% for the same TMP. This confirmed that membranes with higher MWCOs are more sensitive to changes in TMP in terms of total anthocyanin retention and that all the membranes with nominal MWCOs equal to or less than 20 kDa can be used for concentrating anthocyanins of roselle extract.

On model fruit juice solutions, polyphenols that are responsible for haze formation and browning during storage of clear apple juice and concentrates were selectively removed by an ultrafiltration process, at TMP of 1 bar, using tailor-made membranes of polyethersulfone (PES) and polyvinylpyrrolidone (PVP) (Borneman , et al, 2001). Polyphenol removal rates from the model solution were up to 40% for a membrane prepared from a 22.5 % (w/w) polymer solution with a PES:PVP ratio of 3.5. According to the authors, the initial adsorption and flux values could be restored by regenerating the membranes with 0.1 M NaOH.

A solvent extraction method utilizing 50% ethanol and 50% water as solvent was used for the extraction of polyphenols from grape seeds. An additional ultrafiltration step was also included to determine its beneficial effect. Various experimental conditions, such as solid to liquid ratio

(0.1–0.25 g/ml), number of extraction stages (single, double and triple) and membrane pore size (0.22 and 0.45 μm) were investigated to optimize the extraction. The 0.22 μm membrane provided better results than the 0.45 μm membrane due to the fact that the smaller pore size rejected greater amounts of unnecessary particles and solutes. The smaller the membrane utilized, the better the results were, and the purer the polyphenol extract (Nawaz , et al, 2006). According to the authors, the optimal conditions were the extraction of grape seed polyphenols with a 0.2 g/ml solid to liquid ratio, a double stage extraction at TMP of 5 bar and the use of a 0.22 μm membrane pore size. Under these conditions, the maximum amounts of polyphenols (11.4% of the total seeds weight) were recovered from grape seeds (Nawaz , et al, 2006). The UF step played an important role in the extraction process by concentrating the polyphenols.

Galanakis et al, 2013 used UF process for the fractionation of phenolic classes from wine sludge (WS). The protocol consisted primarily to extract phenols in ethanol solution during 1h at 25 ºC. The extraction step allowed the recovery of two extracts which differed in their amounts of sugar, polyphenols and pectins. Afterwards, UF steps with different membrane MWCO (1, 20 and 100 kDa) and material (polysulfone and composite fluoropolymer) were performed on WS extract recovered after alcoholic extraction. According to the authors, polysulfone membranes were able to separate phenolic compounds from pectin fractions, however the different phenolic classes could not be fractionated. Contrary to polysulfone membranes, the fractionation of phenolic classes (hydroxycinnamic acids, flavonols and anthocyanins) by the fluoropolymer membrane was carried out successfully on the basis of polarity, but not in terms of membrane adsorption (Galanakis , et al, 2013).

Ultrafiltration of flaxseed hull extracts was performed to fractionate polyphenols and proteins (Loginov , et al, 2013). After solvent extraction (0.3 M NaOH, 0 wt% ethanol) and centrifugation, extracts of flaxseed hull were purified by protein coagulation, at different pH values, and stirred dead-end ultrafiltration with molecular weight cut-off membrane of 30 kDa and constant TMP of 4.10^5 Pa. According to the authors, the pH value of flaxseed hull extract had a major impact during coagulation and filtration process for the fractionation and recovery of polyphenols and proteins. Indeed, the purity of polyphenols in filtrate after coagulation and centrifugation at pH 4.4, increased from 33.6% to 56.0%, Moreover, it was additionally increased to 76.6% after ultrafiltration.

Green tea (Camellia sinensis L.) contains tea polyphenols with about 10–30% (w/w) polyphenol. These polyphenols include catechines, flavanols, flavanones, phenolic acids, glycosides and the aglycons of plant pigments and are natural antioxidants (Bazinet et al., 2007, Bazinet et al., 2010). To extract the green tea polyphenols, Li et al. (2005) proposed a process integrating an UF step with a cellulose acetate–titanium composite ultrafiltration membrane (CATUFM). After ultrafiltration with the CA–Ti composite ultrafiltration membrane, a product with a tea polyphenol content of more than 40% was obtained. After adsorption on a resin and elution with mixed solvents, a purified product containing more than 90% of tea polyphenol was obtained (Li , et al, 2005). According to the authors, this new technological process that combines CATUFM with adsorptive resins gives an acceptable product without adding any other toxic substances.

Very recently, micellar-enhanced ultrafiltration was used to remove phenols from synthetic waste water (El-Abbassi et al., 2011, Zhang et al., 2012). Gemini surfactants are third generation surfactants that consist of two hydrophilic and hydrophobic head groups, connecting through a spacer group (Meziani et al., 2003, Song et al., 2012). The performance of the selected Gemini surfactant was proved superior on retention of phenol/surfactant (peak value is 95.8% for phenol retention), permeate flux and membrane fouling with respect to other conventional surfactants possessing equal alkyl chain length. These results demonstrated that cationic Gemini surfactant with exceptional structure has favorable prospects in the treatment of phenol wastewater by the micellar-enhanced ultrafiltration (Zhang , et al, 2012).

NF

Cissé et al. (2011) compared the use of UF membrane with ten nanofiltration flat-sheet membranes with MWCOs ranging from 0.15 to 0.4 kDa on the concentration of anthocyanin from roselle. With similar permeate fluxes at average transmembrane pressure, retention of anthocyanins was significantly higher for nanofiltration membranes than for ultrafiltration membranes. For all the NF membranes tested, retention of total anthocyanins ranged between 93% and 100%. These results are consistent with those of several studies that also reported high retention rates for these compounds with the same membranes (Gilewicz-Łukasik et al., 2007). The NF membranes DK and DL from GE Osmonics presented, at 2 MPa of transmembrane pressure, a high potential for concentrating anthocyanins from roselle extracts. Indeed, with applied transmembrane pressure between 2 and 3 MPa, NF membranes presented higher permeate fluxes and retention values – of more than 95% – for total soluble solids, titratable

acidity and anthocyanins. An industrial trial, using a 2.5-m^2 NF filtration surface, on concentration mode showed that a roselle extract can be concentrated from 4 to 25 g total soluble solids per 100 g, multiplying by 6 the anthocyanin concentration. The anthocyanin retention was 100% and no significant damages were observed when comparing concentrate quality with the initial roselle extract.

Machado et al., (2013) studied the concentration of pequi (Caryocar brasiliense Camb.) by NF process for the concentration of its natural antioxidants. The first step consisted to extract polyphenols and carotenoids using double extraction with alcohol (ethanol) or water. The two different extracts recovered (alcoholic and aqueous) were concentrated by NF with membrane MWCO of about 100-300 Da at 25 ºC and under pressure of 800 kPa. The extraction best conditions were 25 ºC during 1 h for aqueous extract and 40 ºC during 24 h for alcoholic extract. After NF experiments, it appeared that the rejection towards carotenoids and polyphenols for alcoholic extract was low with values of 10 and 15%, respectively while high efficiency was obtained for aqueous extract with retention coefficient around 100% and 97% for polyphenols and carotenoids, respectively. These results were explained by solvation of phenolic compounds by alcohol molecules which decrease rejection coefficient (Machado et al., 2013).

Negrão Murakami et al. (2011) used an NF step to extract the highest content of phenolic compounds from Ilex paraguariensis A. Saint Hilaire (mate) a plant from the subtropical region of South America. The extract obtained at 100°C, within 3 min of infusion and at pH 6.0 was submitted to nanofiltration at TMP of 300 kPa, to concentrate the phenolic compounds. All the

phenolic compounds investigated showed abundant concentrations in the concentrate. The gallic acid showed a retention index (R) of 0.95 while the 3,4-dihydroxybenzoic acid and the chlorogenic acid reached an R of 0.99 and 0.98, respectively. Among the phenolic compounds identified in the concentrate at a volumetric reduction factors (VRF) of 4.0, chlorogenic acid and 4,5-dicaffeoylquinic acid, which are compounds that derive from caffeic acid and are present in higher concentrations in mate (Pagliosa et al., 2010), show high antioxidant capacity. This result was better than those observed by Cassano et al. (2009) in the concentration of phenolic compounds through ultrafiltration of Citrus reticulata juice, achieving an R of 0.84. Meanwhile, an R of 0.84 was noted by Mello et al. (2010) in the concentration of phenolic compounds of aqueous propolis extract through nanofiltration. It appeared from these results that the concentration of phenolic compounds increased when the VRF was increased, reaching the highest R of 0.99 when VRF was 4.0. However, the concentration of phenolic compounds was not proportional to the VRF used. Bowen and Doneva (2000) and Tsuru et al. (Tsuru et al., 2001) cited that a number of factors may influence the behaviour of compounds in membrane, as surface morphology, pore size distribution and adhesion in membrane. Further analyses were performed by Negrão Murakami et al. (2013) on Ilex paraguariensis A. Saint Hilaire (mate) concentrated by NF at TMP of 300 kDa in order to evaluate its antioxidant activity *in vitro* and *in vivo*. The mate extract was produced according to the method of Negrão Murakami et al. (2011) and it was concentrated by NF module containing membrane with MWCO ranging between 150 and 300 Da. The results demonstrated that NF fractionation allowed to increase the contents of total phenolic by 338%, the chlorogenic acid by 483%, the theobromine by 323%, the caffeine by 251%, the chlorophyll by 321%, the condensed tannins by 278% and the saponins by 211% in

the concentrated mate extract. Analysis of bioactivity showed that both mate extract and concentrate exhibited antioxidant activity *in vitro* and *in vivo*. However, while concentrated extract was more bioactive than mate extract in the case of *in vitro* tests, it appeared that mate extract gave the best results for *in vivo* tests despite a lower polyphenolic compounds concentration. Consequently, the authors concluded that antioxidant activity does not seem to depend exclusively on total polyphenol content (Negrão Murakami et al., 2013).

The nanofiltration of ethanolic extracts from *Sideritis* spp. L., containing flavonoids and polyphenols, was studied by Tylkowski et al. (2011) with organic solvent resistant membranes of different MWCO (Starmem^{TM} 240 (polyimide), Duramem^{TM} 300 and Duramem^{TM} 500 (both of them modified polyimide)). All membranes used showed high rejection of flavonoids (from 97% up to 99%) as confirmed by the low concentrations observed in the permeate. About 1.62% of the total mass of phenols has passed through the dense active layer of the polyimide membrane Starmem^{TM} 240. In the case of modified polyimide membrane Duramem^{TM} 500 this value was 16.24%, but the rejection of flavonoids remained high (97% vs. 99% for the Starmem membrane). The 300 Da modified polyimide membrane Duramem^{TM} 300 showed complete (over 99%) rejection of both groups of compounds. No distinct effect of pressure on the rejection was observed. Only in the case of Duramem^{TM} 500, increasing the pressure from 30 bar to 50 bar leaded to a slight increase of the rejection. The rejection dependence on the MWCO of the membrane showed that a separation of flavonoids from low molecular polyphenols becomes possible at MWCO >400 Da. Concentrations of active compounds up to 3–4 times higher have been obtained in the retentates. The extracts concentrated by nanofiltration preserve their high

antioxidant activity. The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) radical scavenging activity increases from 84% for the extracts to 99% for the obtained retentates. Further dilution of the retentates displays a logarithmic concentration dependence of the radical scavenging activity.

Conidi et al. (2011) also observed such a high rejection towards polyphenolic compounds during the concentration of bergamot juice. Hence, after NF experiments at TMP of 7.5 bar, the rejection of a 450 Da NF membrane towards flavonoids was in the range 91–99%. Flavonoids were retained in the retentate side of the membrane. The 750 Da NF membrane showed a lower rejection towards flavonoids in the range of 43–62% and a rejection towards polyphenols of 44%. Therefore, phenolic fractions with molecular weight greater than 750 Da were retained in the retentate side of the membrane. Besides, flavonoids identified in the juice (naringin, narirutin, hesperidin and neohesperidin), with molecular weights from 550 to 610 Da, were also partially retained by the membrane (Conidi et al., 2011). The rejection towards sugars of the 450 Da NF membrane was of about 48% indicating that this membrane has the best performance in terms of separation between sugars and flavonoids in the clarified juice (Conidi et al., 2011). The same authors used nanofiltration on orange press liquor for the recovery of phenolic compounds (Conidi et al., 2012). In this context, four NF membranes characterized by different MWCOs (180, 300, 400 and 1000 Da) and material were used. After fractionation at a TMP of 20 bar, the results demonstrated that the rejection toward anthocyanins was quite similar whatever the NF membrane MWCO. However, concerning flavonoids, it was shown that the more membrane MWCO was important, the more the rejection value decreased. Concerning the rejection towards

sugars, the same tendency as described for flavonoid was observed but with a greater decrease especially for the 1000 Da NF membrane (rejection of 22.8%). Consequently, the authors concluded that the NF membrane with a MWCO of 1000 Da demonstrated the best performances for the separation of phenolic compounds (rejection towards anthocyanins and flavonoids of about 89.2 and 70%, respectively) and sugars (22.8%).

Integrated filtration processes

The use of integrated membrane systems is becoming another real alternative to recover polyphenols as it is established in some recent works.

In particular, Paraskeva et al. (2007) found that the olive mill wastewater (OMW) may be treated efficiently by using UF, NF and/or RO to obtain a permeate fraction which can be discharged in aquatic systems according to national or EU regulations or to be used for irrigation. In this case NF was employed for the separation of the most part of phenols present. A MF/UF/RO membrane process for the selective fractionation and total recovery of polyphenols from OMW was also proposed by Russo (2007). It was based on the preliminary MF of the OMW, followed by two UF steps realised with 6 kDa and 1 kDa membranes, respectively, and a final RO treatment. The RO retentate, containing enriched and purified low molecular weight polyphenols, was proposed for food, pharmaceutical or cosmetic industries while MF and UF retentates can be used as fertilizers or in the production of biogas in anaerobic reactors. Recently, Garcia-Castello et al. (2010) proposed an integrated MF/NF membrane system for the recovery, purification and concentration of polyphenols from OMW. The proposed system included MF

and NF. The OMW was directly submitted to a MF operation without preliminary centrifugation. This step allowed to achieve a 91% and 26% reduction of suspended solids and total organic carbon (TOC), respectively. Moreover, 78% of the initial content of polyphenols was recovered in the permeate stream. The MF permeate was then submitted to a NF treatment. Almost all polyphenols were recovered in the produced permeate solution, while TOC was reduced from 15 g/L to 5.6 g/L. A recent study tested the potential of an integrated membrane system in the treatment of OMW for the recovery of purified fraction enriched in polyphenols (Cassano et al., 2013). The first step consisted in submitting the OMW to an UF operation (nominal pore size of 0.02 μm) to remove suspended solids and to reduce fouling phenomena. The UF permeate recovered was treated again by UF with MWCO membrane of 1000 Da for the concentration of polyphenols. Finally, the second UF permeate was treated by NF to separate water and solute fraction. The rejections of both UF membranes towards total polyphenols were in the range 26.7-31.8%. Concerning NF membrane it reached 93%. However, while rejection values for low molecular weight (LMW) polyphenols were not important after UF fractionations (2 and 18%), NF step allowed the rejection of 100% of these molecules. Consequently, the successive step of pressure-driven membrane processes for the treatment of OMW allowed the recovery of a concentrated solution enriched in polyphenolic compounds suitable for cosmetic, food and pharmaceutical industries.

Integrated processes were also used for fruit juice polyphenols extraction. A depectinised bergamot juice was clarified by using a 100 kDa polysulphone hollow fibre (HF) membrane module. The clarified juice was then treated with UF and NF membranes characterised by

molecular weight cut-off (MWCO) of 1000, 750 and 450 Da (Conidi et al., 2011). The results obtained by the authors suggest that an integrated membrane process based on the preliminary UF of the depectinised juice with a 100 kDa membrane, followed by a NF step with a 450 Da membrane, seems to be the best procedure for the separation of polyphenols contained in the bergamot juice and to obtain fractions with different phenolic content and different antioxidant activity. The UF pretreatment of the juice is essential in removing suspended solids and reducing fouling phenomena in the following NF step while phenolic compounds are recovered in the retentate stream of the NF process. The phenolic compounds are partially separated from the sugar since around 52% of sugar is recovered on the permeate side (Conidi et al., 2011).

Integrated processes could also be used for plant polyphenol extraction and concentration. Indeed, ultrafiltration steps were used in the works of Diaz-Reinoso et al. (2011). Thus, aqueous extraction of Castanea sativa leaves (CsL) was scaled up and the extract was processed in a series of two UF membranes (5 and 10 kDa) with the aim of concentrating the active phenolic compounds with antioxidant activity (Diaz-Reinoso et al., 2011). The authors tested two different configurations; a sequential filtration with a batch dilution of the retentate and a sequential filtration without retentate dilution. The different UF treatments were performed at a TMP of 20 bar. The sequential filtration without dilution of the 5 kDa permeate provided a selective separation of active compounds on 10 kDa membrane filtration process. The final concentrated extracts presented radical scavenging capacities comparable to Trolox and to BHA.

Koffi et al. (2013), used MF and RO for the extraction of phenolic compounds from *Justicia secunda* Vahl leaves. After soaking plant leaves in an ultrasonic pilot plant, the first step consisted to filter the water extract by MF with pore size of 0.2 µm, under TMP of 0.6 bar with a retentate tangential velocity of 5 m/s. The second step consisted to recover the MF permeate and to concentrate it by RO at a constant TMP of 40 bars. The authors showed that the multi-step process allowed the concentration of active polyphenol compounds by a factor 19 compared to the simple beverage obtained by water diffusion of dried leave (Koffi et al., 2013).

ED

Polyphenols in tobacco are known as precursors for bioactive phenolic compounds in cigarette smoke. Electrodialysis has been tested as a means of selectively extracting polyphenols from aqueous tobacco extracts (Bazinet et al., 2005). Seven commercial membranes were tested in an electromigration configuration. This exploratory study showed the potential application of AM-2 and AM-4 membranes for the migration of polyphenols. During 3-h trials with a single membrane in an electrodialysis cell, both AM-2 and AM-4 membranes achieved a 77% demineralization rate. The migration rates for the AM-2 and AM-4 membranes were for chlorogenic acid 24.7 and 28.7%, respectively, for scopoletin 8.6 and 18.8%, and for rutin −10.3 and 10.3% (Bazinet , et al, 2005). These low migration rates obtained for polyphenols were not sufficient to demonstrate that it was a practical technology. Consequently, a second work was carried-out with membrane stacking for more representative industrial conditions (Bazinet et al., 2005). Hence, three anionic membranes of the same type, AM-4 or AM-2, were stacked in a cell

and the duration of treatment was extended to 4 h. Trends previously observed during electromigration treatments for these membranes were confirmed. High polyphenol migration rates were obtained with these membranes: for the AM-4 and AM-2 membranes the rates were, respectively, for chlorogenic acid 90.8 and 86.5%, for scopoletin 66.7 and 23.2%, and for rutin 81.3 and 31.1%. These results confirmed that ED has the potential for efficient separation of tobacco polyphenols.

EDFM

EDFM a new electrically driven technology has been recently tested for the recovery of polyphenols. This technology is a sustainable and environmentally friendly technology and appears to be a one-step process. Another advantage of this technology applied to fruit juice is that compounds of interest would be directly transferred from one juice to another without the need for previous solvent extraction, and without sugar concentration or migration (Bazinet , et al, 2009). EDMF was used on green tea to perform selective extraction of catechins and on cranberry juice to enrich cranberry juice in its own natural phenolic antioxidant compounds.

Concerning green tea, the aim of this exploratory study, carried-out by Labbé et al. (2005) was to evaluate the feasibility of selectively extracting catechins from a green tea solution using an electrodialysis cell. Commercially available membranes (AMX-SB, AFN, PC-400D and UF-1000 Da) were tested for their potential to allow migration of green tea catechins. This study demonstrated that epigallocatechin (EGC) and EGCG from a green tea infusion can migrate at a

high rate through an electrodialysis (ED) system. Among tested membranes, the UF-1000 Da membrane can achieve an EGC and EGCG migration as high as 50%. The other studied catechins and caffeine had no significant migration rate through either the anionics or the UF membranes. Thus, this method combined to a previously developed EGCG preconcentration procedure might allow the production of a green tea extract with highly active biological compound.

Recently, Bazinet et al. (2009) have demonstrated that it is possible to enrich a cranberry juice in antioxidants from another cranberry juice by electrodialysis with filtration membrane (EDFM). In this application, a volume of cranberry juice was circulated in both compartments on each side of the filtration membrane: a large volume from which the antioxidants migrate and a smaller volume to be enriched. In this study, it was shown that the total concentrations of proanthocyanidins and anthocyanins increased by 34.8% and 52.9%, respectively, in cranberry juice treated with the EDFM system. In parallel, an 18% increase of the antioxidant capacity of the enriched cranberry juice was obtained by the EDFM treatments. Moreover, the taste of the enriched cranberry juice was improved in comparison with the non-treated juice. Based on these results the authors concluded that the production of phenolic antioxidant enriched cranberry juice could be feasible at a large scale and proposed an integrated process flow. According to this process flow, the EDFM process would be directly connected to bottling process of cranberry juice to produce antioxidant enriched-cranberry juices in batch process and cranberry juice with very low variation in antioxidant in a continuous process. An advantage of this technology is that compounds of interest would be directly transferred from one juice to another without the need

of solvent to previously extract them (Bazinet et al., 2009). In order to transpose this technology on an industrial scale for the production of an antioxidant enriched cranberry juice, Bazinet et al.(2012) tested the integration of EDFM to the conventional process used for cranberry juice production, in a way avoiding the generation of a source juice impoverished in polyphenols. In this second study, the anthocyanin concentrations and the antioxidant capacity of the enriched juice increased respectively of 19.4% and 23.7%, while the anthocyanin concentrations and antioxidant capacity of the raw juice remained constant throughout treatments. Proanthocyanidin concentrations of both juices remained also constant which suggests that the duration of treatments was too short to allow the migration of these molecules (Bazinet , et al, 2012). To further understand the effect of UF membrane MWCO and material during EDFM of cranberry juice, Husson et al. (2013) tested six different UF membranes for their capacity to enrich juice in anthocyanins. In this context, 3 PES (0.1 µm, 500 and 10 kDa), 2 PVDF (100 and 150 kDa) and 1 PS (10 kDa) UF membrane were choosen. After EDFM experiments, the authors demonstrated that the higher enrichment yields in anthocyanins were obtained with 150 kDa PVDF and 500 kDa PES UF membranes with value of 24%. It was also demonstrated that anthocyanin migration was influenced by the UF membrane zeta potential since UF membrane with negative charges seems to facilitate the electromigration of molecules due to the attraction of opposite charges (Husson et al., 2013).

The concentration and recovery of phenolic compounds was subjected to successive steps which include preparation of the raw material, its fractionation by pressure-driven and/or electrically drivenmembrane processes and determination of the fraction composition (Fig. 9).

The different studies, listed in this section, described fractionation experiments performed with both liquid and solid samples. The liquid raw material is generally a lipid solution such as crude oil, or a solution containing water as a main solvent such as fruit juices. Concerning fruit juices, a step of depectinization by using enzyme (pectinase) is necessary to remove larger particles and to increase phenolic recovery yield. Concerning solid raw material, successive steps are performed before the membrane fractionation until obtaining a liquid extract. Indeed, a sample treatment by organic solvents (hexane, alcohols) or by water allows the extraction and the recovery of phenolic compounds from solid material. Afterwards, a step of filtration or centrifugation is performed to separate solid extract, which is not retained, from liquid extract, recovered to be separated. After the fractionation step(s) (by MF, UF, NF, ED, EDMF or integrated membrane process) and the recovery of phenolic fractions, the sample composition is analyzed by HPLC and antioxidant activity could be evaluated.

Ascorbic, isoascorbic, folic and citric acids

The richest natural sources of ascorbic acids and its derivatives, as well as other organic acids are fruits and vegetables. Recently, due to the presence of organic acid having antioxidant properties and exerting positive effects on human health there is a growing interest in natural products. In this context, different membrane technologies were developed to extract or concentrate these antioxidants compounds.

MF

Laorko et al. (2010) during their experiments on microfiltration (membrane pore size of 0.1 and 0.2 μm and TMP of 0.7 bar) of pineapple juice also measured the ascorbic acid content of the treated juice. As for the polyphenols, the 0.2 μm membrane gave the highest total vitamin C content.

UF

The pressure-driven membrane separation of dilute single-solute aqueous solutions of ascorbic and citric acids amongst other compounds was first studied by Thiel and Lloyd (1983) at operating pressures ranging from 100 to 400 kPa, using an asymmetric cellulose acetate membrane in a batch stirred cell. Results were analyzed based on a mass transfer analysis which included both convective and diffusive effects. Rejection followed the trend glucose ≈ mannitol ⩾ ascorbic acid ⩾ citric acid. Transport parameters followed the trend glucose ≈ mannitol < ascorbic acid < citric acid. The observed results are explained on the basis of significant physicochemical interactions between solute, solvent, and membrane.

Cassano et al. (2008) studied the influence of ultrafiltration (UF) on the composition of ascorbic acid of the kiwifruit juice in order to develop a natural product which can be used to fortify foods and beverages. As mentioned previously for phenolics compounds, the optimal TMP value occurred at 0.6–0.65 bar with temperature in the range 20–30 °C. The recovery of folic, ascorbic and citric acids, in the clarified juice, with respect to the initial feed, was dependent on the final

VRF of the UF process: an increase of the VRF determines an increase of these compounds in the clarified juice. The rejections of the UF membrane towards these compounds were in the range 0–4.3%. The authors concluded that the limiting factor for recovering these antioxidant compounds in the clarified juice fraction is the final VRF of the process: an increase of this parameter allows a higher recovery of these compounds in the permeate stream. The lower recovery observed for the ascorbic acid, if compared with the other investigated acids, can be attributed to a higher degradation of this compound as confirmed also by the mass balance of the UF process. Similar experiments were carried-out by the same research team on kiwi fruit. Fresh depectinised kiwifruit juice was clarified by ultrafiltration (UF) process. UF experiments were carried-out in batch concentration mode and performed at a TMP of 0.85 bar, an axial feed flow rate of 800 l/h and a temperature of 25°C (Cassano et al., 2006). Only a slight decrease of the total antioxidant activity (~4%) and of the ascorbic acid (~0.5%) was observed in the permeate with respect to the fresh juice. By a 8% reduction of the total antioxidant activity was observed in the concentrated juice at 61.4°Brix with respect to the UF permeate (Cassano , et al, 2006). The reduction can be attributed to a 16% degradation of the initial content of ascorbic acid (Cassano et al., 2007). The authors proposed the addition of a degassing treatment before UF to drastically reduce this phenomenon (Cassano , et al, 2008).

ED

Vera Calle et al. (2003) were indirectly precursor in the use of ED cells for the recovery of citric acid, since their final aim was to deacidify clarified passion fruit juice, *Passiflora edulis* f.

flavicarpa. The ED deacidification was performed with a laboratory cell of 20 cm^2 of effective area at a constant current density of 400 A/m^2. The principle of the ED configuration was the extraction of citrate anions from the juice and their replacement by hydroxyl ions provided either by the NaOH solution in the C2 compartment (ED3C configuration, Fig. 10). In all the deacidified juices, a decrease in the citrate and malate concentrations was obtained. In the electrodialysis method, the elimination of anions was related to their mobility both in the solution and membrane, and consequently the extraction of inorganic anions was easier than that of organic anions.

EDBM

Vera Calle et al. (2002) also tested the deacidification of clarified passion fruit juice, *Passiflora edulis* f. *flavicarpa*, by electrodialysis with bipolar membrane (EDBM). The stack was equipped with homopolar and bipolar membrane, forming two compartments (EDBM2C configuration, Fig. 11). The reduction of acidity was achieved by increasing the pH from 2.9 to 4.0. In this EDBM configuration only anions are able to pass through the anion-exchange membrane from the juice to the concentrate (C) compartment. The net effect was the extraction of anions, mainly citrate, and their replacement by hydroxyl ions provided by the BPM. Citric acid was formed in the concentrate (C) compartment by citrate ions extracted from juice and protons provided by the second BPM separating the (C) compartment and electrode compartments. This configuration allowed the production of citric acid with 89% purity and avoids the increase in the sodium concentration in the juice.

In a further study, the previous configuration was compared with a modified one, in which the stack was equipped with homopolar and one bipolar membrane constituting three compartments (EDBM3C configuration, Fig. 12) (Vera Calle et al., 2003). They observed that the deacidification rates and current efficiencies were similar for both processes. However, although the non-modified configuration induces the greatest energy consumption (0.50 vs 0.38 kWh/L of juice), it offers the advantage of eliminating the consumption of chemicals. The inorganic ions were almost eliminated, 62 % of the citrate ions and 48 % of the malate ions were removed from the fresh juice to produce citric and malic acids. ED with bipolar membrane could be a promising alternative to the conventional calcium precipitation process for the deacidification of the passion fruit juice and the production of citric acid.

EDFM

During EDFM of cranberry juice, Bazinet et al. (2012) observed that enriched juice concentrations in citric and malic acids decreased respectively of 7.3% and 4.7% resulting in a significant decrease of its sour taste intensity, a major problematic for cranberry juice producer. However, the concentration in the raw juice was not significant due to the high ratio raw juice/enriched juice.

The raw materials generally used for ascorbic, isoascorbic, folic and citric acids concentration are fruit juices such as kiwi, passion or cranberry fruit juices. Concerning the experiments of Thiel and Lloyd (1983), the author used a model solution containing ascorbic and citric acids molecules. Concerning fruit juices, filtration studies are performed on raw juice or depectinized juice. Afertwards, sample fractionations are carried-out by pressure-driven membrane processes (MF or UF), however, most experiments described above used electromembrane processes (ED, EDBM or EDFM). Finally, fraction composition is analyzed by HPLC to determine the effectiveness of filtration process for the concentration of target molecules (Fig. 13).

Peptides

Numerous studies have described the antioxidant activity of peptides generated from the hydrolysis of various proteins, such as soy protein (Chen et al., 1995, Lee et al., 2008), whey protein (Contreras et al., 2011), casein (Suetsuna et al., 2000, Kansci et al., 2004, López-Expósito et al., 2007, Gómez-Ruiz et al., 2008), egg-white protein (Dávalos et al., 2004), meat and fish proteins (Carlsen et al., 2003, Je et al., 2005, Mendis et al., 2005, Je , et al, 2007), potato (Pihlanto et al., 2008), and gelatine obtained from skin of sole and squid (Giménez et al., 2009) (Table 2). In general, a range of different processes are used to isolate and purify bioactive peptides from protein hydrolysates. Membrane separation techniques such as nanofiltration (NF), ultrafiltration (UF) and electro-membrane filtration provide a potentially suitable industrially relevant technology for the enrichment of peptides within specific molecular weight ranges (Bourseau et al., 2009, Picot et al., 2010, Harnedy and FitzGerald 2012).

Enzymatically hydrolyzed peptides exhibit different physicochemical properties and biological activities depending on their molecular weight and amino acid sequence. Therefore the molecular weight and the charge (according to the pH) of the bioactive peptide is one of the most important factors in producing bioactive peptides with the desired biological activities (Kim and Mendis 2006, Kim and Wijesekara 2010). In general, bioactive peptides are separated into fractions using membranes in the range 1–10 kDa (Jeon et al., 1999, Je , et al, 2005, Rajapakse , et al, 2005). According to their Molecular weight cut-off (MWCO), Ultrafiltration (UF) and/or nanofiltration (NF) can be used to refine hydrolysates and also to fractionate them in order to obtain a peptide population enriched in selected sizes. NF can be used to concentrate hydrolysates (Fenton-May et al., 1971, Tessier , et al, 2006, Vandanjon et al., 2007) whereas UF membranes with high MWCO (20 to 100 kDa) are adapted to the separation of peptides and non-hydrolyzed proteins or proteolytic enzymes (Bouhallab and Touzé 1995, Lajoie et al., 2001). On the other hand, UF membranes with intermediate MWCO (about 4000 to 8000 Da) allow hydrolysates to be fractionated with the result of enrichment in some ranges of molecular weight (Vandanjon , et al, 2007). Very recently, a combination of UF membrane stacked in a electrodialysis cell have was tested successfully for the selective separation of anionic and cationic antioxidant peptides (Langevin et al., 2012).

UF

Different marine and animal sources hydrolysates (saithe, sea urchin gonads, cod frames, shrimp, bivalve mollusc, blue and Korean mussel, monkfish and egg yolk) were produced and separated by UF for the production of antioxidant peptides. Chabeaud et al. (2009) tested five organic tubular ultrafiltration membranes of various material and molecular weight cut-off (MWCO) for the enrichment of a fish (saithe, Pollachius virens) hydrolysate in low molecular weight antioxidant peptides (<2 kDa). The fractionation was performed at 60 °C and low TMP (1 to 5 bar). Two membranes exhibited interesting selectivity characteristics. The 4 kDa m-PES membrane had a global retention factor in peptides of about 67% (5 bar) and produced the most enriched permeate in peptides lower than 2 kDa. The 8 kDa PS membrane exhibited high permeation flux, retained (almost) totally large peptides but was highly permeable to small and medium-size peptides, appearing thus likely to be used in sequence with the 4 kDa PES to recover medium-size peptides. Finally, a better separation between peptides larger and smaller than 2 kDa was obtained when the solution was ultrafiltered at low pressure, meaning that the MWCO of a membrane can be adjusted according to fractionation objective by acting upon the pressure (Chabeaud , et al, 2009). Purple sea urchin (Strongylocentrotus nudus) gonad was treated separately with neutral protease, papain, pepsin and trypsin. The resultant hydrolysates were fractionated using a series of ultrafiltration steps (MWCOs of 10, 5, 3 and 1 kDa) (Qin et al., 2011). Five fractions were prepared from each hydrolysate and the corresponding molecular weight ranges were below 10 kDa, 5-10 kDa, 3-5 kDa,1-3 kDa and below 1 kDa. Results indicated that all peptide fractions possessed DPPH radical scavenging capacity and reducing

power in a dose-dependent manner. For all four hydrolysates, the below 1 kDa fractions exhibited the highest DPPH radical scavenging capacity. The below 1 kDa fractions prepared with neutral protease, papain and pepsin, and the 1-3 kDa fraction prepared with trypsin showed the highest reducing capacity among corresponding hydrolysates (Qin , et al, 2011). Fractions below 10 kDa prepared with all four proteases were found to have the ability of scavenging DPPH radical in a dose dependent manner at the concentration of 1.25-10 mg/mL (protein basis). The scavenging effects of these four fractions below 10 kDa were significantly lower than that of Vit-C ($P < 0.05$) but close to or stronger than that of other protein hydrolysates (Chen et al., 2007, Wang et al., 2008, Yang et al., 2008). A number of studies have demonstrated a good correlation between certain amino acid residues and antioxidant ability of protein hydrolysates (Saiga et al., 2003). For example, peptides with high contents of histidine, alanine, valine, methionine, and leucine have been reported to possess strong antioxidant capacity (Chen et al., 1996, Guo et al., 2009). As containing all such antioxidant related amino acid residues, the below 10 kda fractions would be expected to exhibit strong antioxidant ability.

Hoki or cod frame protein, which is normally discarded as an industrial by-product in fish plants, was hydrolyzed with various enzymes and treated by different UF steps (Je et al., 2005). The antioxidative activity of the hydrolysates was investigated, and the results showed that pepsin hydrolysate has the highest activity. Hoki frame protein hydrolysates (HPH) prepared by pepsin were fractionated according to the molecular mass into four major types, HPH I (5–10 kDa), HPH II (3–5 kDa), HPH III (1–3 kDa), and HPH IV (below 1 kDa), using an ultrafiltration membrane. HPH III, which permeated the 3-kDa membrane but not the 1-kDa membrane, showed the highest antioxidative activity at 0.5 mg/ml and the highest scavenging effects for

DPPH and superoxide anion radicals. Addition of HPH III suppressed 80% of the hydroxyl radical at 0.5 mg/ml. The results showed that HPH III effectively quenched alkyl radical by over 85% at 1 mg/ml. The results showed that HPH III has the highest antioxidative activity, and that the activity was dependent on the molecular weight (Je , et al, 2005). Similar experiments were carried-out by Jeon et al. (1999) with membrane having different MWCOs. Hence, a cod frame protein hydrolysate (CFPH) was processed through a series of ultrafiltration (UF) membranes with MWCOs of 30, 10, 5 and 3 kDa (Jeon et al., 1999). Four types of permeates including 30-K (permeate from 30 kDa), 10-K (permeate from 10 kDa), 5-K (permeate from 5 kDa) and 3-K hydrolysate (permeate from 3 kDa) were obtained. The 10-K hydrolysate possessed the most effective antioxidative activity and showed approximately twofold higher activity than the original hydrolysate. The activity was also as high as that of alpha-tocopherol. According to Jeon et al. (1999), the hydrolysates separated using UF membranes showed some advantages including mass production of the desirable fractions and enhancement of some functionalities in comparison to the original hydrolysate, together with simplification of the separation process and reduction in the cost of production compared to chromatographic processing

On shrimp processing byproduct, Zhao et al. (2011) used four UF membranes (10, 5, 3 and 1 kDa) to separate an alcalase hydrolysate and to produce a peptide fraction with antioxidant properties. The protein content, DPPH radical scavenging activity and molecular weight of each fraction were determined. The results showed that the hydrolysate was composed with high amounts of hydrophobic amino acids (40.4%) which might contribute to the high antioxidant activity. The fraction with MW lower than 1 kDa exhibited the highest antioxidative activity among the five fractions.

Recently, Kim et al. (2013) studied the antioxidant capacity of a bivalve mollusc named Ruditapes philippinarum (R. philippinarum). After enzymatic hydrolysis by various enzymes, protein hydrolysate was fractionated by different MWCO UF membranes (30, 10 and 5 kDa). Four different fractions were recovered: >30 kDa, 10-30 kDa, 5-10 kDa and <5 kDa and were tested for their antioxidant activity. The authors determined that the <5 kDa fraction exhibited the highest hydroxyl radical scavenging activity. After successive steps of < 5 kDa fraction purification, the antioxidant peptide sequence was identified as Ser-Val-Glu-Ile-Gln-Ala-Leu-Cys-Asp-Met (Kim et al., 2013).

Blue mussel (*Mytilus edulis*) protein hydrolysate was investigated by Wang et al. (2013) for the determination of its antioxidant activity. The protocol consisted to hydrolyze blue mussel protein by various enzymes (neutrase, alcalase, pepsin and papain) during 4 h and to test DPPH scavenging activities of generated hydrolysates. The first results showed that neutrase hydrolysate (NH) recovered after 3 h of hydrolysis demonstrated the highest DPPH scavenging activities (28.8 ± 1.79% at a hydrolysate concentration of 10 mg/mL). Consequently, only NH was fractionated by UF with membrane MWCO of 3 and 10 kDa. Consequently, three peptide fractions (NH-I, NH-II, and NH-III) were obtained (>10, 3–10, and <3 kDa, respectively) and were tested for their DPPH radical scavenging activities. The DPPH radical scavenging activities of NH-I, NH-II and NH-III were 12.62 ± 1.99%, 21.88 ± 2.71%, and 35.7 ± 2.01%, respectively at the concentration of 10 mg/ml. Thus, NH-III fraction showed higher antioxidant activity than the other two fractions. Purification of NH-III fraction by gel filtration chromatography and RP-HPLC allowed the recovery of a novel antioxidant peptide identified as YPPAK (574 Da). This peptide exhibited good scavenging activity on DPPH radical, hydroxyl radical, and superoxide

anion radical with EC50 of 2.62, 0.228, and 0.072 mg/ml, respectively. The purified peptide was also effective against lipid peroxidation in a linoleic acid model system (Wang et al., 2013). The antioxidant activity of *Mytilus coruscus* mussel was studied by Kim et al. (2013). First, fresh mussels were hydrolyzed by eight different enzymes and radical scavenging activities of various enzymatic hydrolysates were tested. Authors determined that hydrolysate obtained after papain digestion exhibited the strongest antioxidant activity and was therefore selected for further analysis. In the same manner as above, ultrafiltration was performed with membrane MWCO of 30, 10 kDa and 5 kDa which generated four peptide fractions (> 30 kDa (MWCO I), 10-30 kDa (MWCO II), 5-10 kDa (MWCO III) and < 5 kDa (MWCO VI)). Among the four MWCO fractions, MWCO I fraction, showed the highest hydroxyl radical scavenging activity (IC_{50} of 0.368±0.053 mg/mL) and was consequently selected for purification steps. Finally and after successive chromatographic purifications, a novel antioxidant peptide was obtained, and the sequence was identified as Ser-Leu-Pro-Ile-Gly-Leu-Met-Ile-Ala-Met (Kim et al., 2013).

Monkfish muscles were hydrolyzed with trypsin and generated hydrolysate was filtered using two UF membranes with MWCO of 1 and 3 kDa (Chi et al., 2014). Thus, three fractions with MW of 3 kDa (MPH-I), 1-3 kDa (MPH-II), and <1 kDa (MPH-III) were recovered and tested for their hydroxyl radical scavenging activity. The low MW peptide fraction (MPH-III) demonstrated the highest hydroxyl radical scavenging activity whatever the hydrolysate concentrations tested (3, 5 and 10 mg/mL). Consequently, only MPH-III fraction was purified by gel filtration chromatography and RP-HPLC. Purification steps allowed the recovery of three antioxidant pentapeptides (Glu-Trp-Pro-Ala-Gln, Phe-Leu-His-Arg-Pro and Leu-Met-Gly-Gln-

Trp). Moreover these three peptides exhibited good scavenging activities on hydroxyl radical (EC_{50} 0.269, 0.114 and 0.040 mg/ml), DPPH radical (EC_{50} 2.408, 3.751, and 1.399 mg/ml), and superoxide anion radical (EC_{50} 0.624, 0.101, and 0.042 mg/ml). Authors concluded that the antioxidant activities were due to the small sizes of peptides and the presence of antioxidant and hydrophobic amino acid residues within their sequences.

Recently, on egg sources, Chay Pak Ting et al. (2011) compared the antioxidant activity of two distinct hydrolysates and their peptide fractions prepared by ultrafiltration (UF) using membranes with molecular weight cut-off of 5 and 1 kDa. The hydrolysates were: 1) a delipidated egg yolk protein concentrate (EYP) hydrolyzed with a combination of two bacterial proteases, and 2) a phosphoproteins (PPP) extract partially hydrolyzed with
trypsin. The ORAC values were low for EYP and PPP hydrolysates with values of 613.1 and 489.2 μM TE/g of protein, respectively. UF-fractionation of EYP hydrolysate increased slightly the antioxidant activity in permeate fractions (720.5–867.8 μM TE/g of protein). However, ORAC values were increased by more than 3-fold in UF-fractions prepared from PPP hydrolysate. These fractions were enriched in peptides with molecular weight lower than 5 kDa. These UF-fractions contained high amounts of histidine, methionine, leucine and Phenylalanine, which are recognized as antioxidant amino acids, but also high content in lysine and arginine which both represent target amino acids of trypsin used for the hydrolysis of PPP. Other experiments were performed on hen egg white lysozyme subjected to a simulated gastrointestinal digestion to determine functional bioactivities of enzymatic hydrolysates generated (Rao et al., 2012). After gastrointestinal digestion, two different hydrolysates were recovered: a peptic

(LPH1) and an α-chymotrypsin and trypsin (LPH2) hydrolysates. Two additional samples were obtained after treatment of LPH1 and LPH2 by ultrafiltration with a 3 kDa cut-off membrane. Results demonstrated that LPH2 hydrolysate had in vitro ACE inhibitory and antioxidant activities. Moreover, UF step significantly improved the antioxidant activity of the LPH2. Further analysis on LPH2-3 kDa composition revealed that the hydrolysate was composed of 38 peptides which several fragments identified as KVF, MKR, AMK, AKF, RGY, WIR, VAW and GIL showed great ACE inhibitory activities. Tanzadehpanah et al. (2012) studied Ostrich (Struthio camelus) egg white (OEW) protein hydrolysates to demonstrate their antioxidant activity. Indeed, OEW proteins were first hydrolyzed by various enzymes (α-chymotrypsin, pepsin, trypsin and papain) and it was demonstrated that tryptic hydrolysate exhibited the highest antioxidant activity. Afterwards, tryptic hydrolysate was treated by ultrafiltration with a 3 kDa cut-off membrane in order to concentrated low MW peptides (fractions with less than 3 kDa molecular mass). Filtrate was purified using RP-HPLC and eight peptide fractions (F1-F8) were separated and their antioxidant activities were tested. The results showed that the F6 fraction possessed the highest antioxidant activity due to a peptide sequence identified as LTEQESGVPVMK.

UF was also used on vegetal sources such as corn, soybean, alfalfa, potato, cowpea, rapeseed, red date, rice, mungbean, and canola. Corn protein was hydrolysed by three microbial proteases and further separated by sequential ultrafiltration (Zhou et al., 2012). The collected protein hydrolysates were diluted with water and ultrafiltered through a 10 kDa membrane under 40 psi nitrogen gas to afford two fractions: retentate (F1, represented hydrolysates > 10 kDa) and

permeate (MW < 10 kDa). The permeate was further ultrafiltered through a 3 kDa membrane to obtain the second retentate (F2, represented hydrolysates between 3 and 10 kDa) and permeate. The permeate was further ultrafiltered through a 1 kDa membrane to yield the third retentate (F3, represented hydrolysates between 1 and 3 kDa) and permeate (F4, represented hydrolysates < 1 kDa). The oxygen radical absorbance capacity (ORAC) of the hydrolysates varied significantly between 65.6 and 191.4 mmoles Trolox equivalents (TE)/g dried weight with a small peptide fraction (NP-F3) produced by neutral protease (NP) possessing the highest antioxidant activity. The DPPH scavenging activities of the hydrolysate fractions also varied significantly between 18.4 and 38.7 μmoles TE/g. Two fractions (AP-F2 and AP-F3) produced by alkaline protease (AP) showed the strongest activity. However, no significant difference was detected on the chelating activity of the fractions. NP-F3, AP-F2, and AP-F3 were incorporated into ground beef to determine their effects on lipid oxidation during 15-day storage period. NP-F3 was the only fraction that inhibited lipid oxidation at both 250 and 500 μg/g levels by as much as 52.9%. The recovery rate of NP-F3 from corn protein isolate was 6.3%, suggesting that it could be practically generated and used in food products to delay lipid oxidation. Similar results were obtained previously by the same research team in the same condition of operations on soy protein hydrolyzate (Zhang et al., 2010). All the 12 hydrolysate fractions showed noticeable ORAC values but varied from 23.8 to 83.8 μmol Trolox equivalents (TE)/g. The hydrolysates also possessed significantly different DPPH scavenging activities and transition metal chelating activities. Three fractions with strong antioxidant activities (NP-F1, Val-F1, and AP-F3) were tested into ground beef: AP-F3 and NP-F1 significantly reduced meat lipid peroxidation by 20.1% and 12.9%, respectively (Zhang , et al, 2010).

On soybean hydrolysate, Moure et al. (2006) showed that UF soy peptides fractions with a molecular weight lower than 10 kDa were the most effective as antioxidants. This result could be correlated with the highest concentration of phenolic amino acids (AA) in permeate fractions, such as tyrosine and phenylalanine, that could act as antioxidant compounds (Moosmann and Behl 2000, Stadtman and Levine 2003). In a recent study on the screening of *in vitro* bioactivities of a soy protein hydrolysate separated by hollow fiber and spiral-wound ultrafiltration membranes, it was demonstrated that UF membrane configuration and treatment time influenced fractions composition, and consequently, their potential antioxidant properties (Roblet , et al, 2012). Hence, it appeared from these results that Tyrosine, Phenylalanine and Leucine residues were more present in permeates, whatever the type of membrane configuration used and, consequently, the treatment duration. Since these AA were concentrated in permeates, the authors concluded that they were part of small or very small molecular weight peptides under 1000 Da or simply AA residues alone. The presence of these small peptides rich in these AA residues or AA residues alone affected mainly the antioxidant capacity results. In fact UF permeate fractions offered the best response to ORAC assays, with an enhancement of antioxidative activity for SWP and HFP of respectively 65% and 75% compared to control demineralized hydrolyzate (Roblet , et al, 2012). These ORAC test results can be related to the presence of these AA and mainly Phenylalanine and Tyrosine. Indeed, Tyrosine and Phenylalanine possess a phenol group on their structure and, being prime targets for oxidation by various forms of reactive oxygen species (ROS), are well known for their antioxidant capacity (Moosmann and Behl 2000, Stadtman and Levine 2003).

An Alfalfa leaf protein was hydrolysated and further fractionated by ultrafiltration by Xie et al. (2008). At a concentration of 35 mg/mL, the alfalfa leaf protein hydrolysate was fractionated through an ultrafiltration membrane system with a 3000 Da UF membrane: The ultrafiltration was performed with a polysulphone (PS) membrane at 0.30 MPa and 20 °C. The DPPH scavenging activities on radical and superoxide radical of alfalfa leaf peptides (ALPs) were respectively 79.7% at 1.60 mg/mL and 67.0% at 0.90 mg/mL. In addition, ALPs showed a 65.1% chelating effect on ferrous ion at 0.50 mg/mL. The molecular weight of the peptides was determined and 67.9% of the total amount was below 1000 Da.

Proteins were isolated from potato tubers (Solanum tuberosum) at different physiological states, and by-products from the potato industry were used to evaluate their radical-scavenging potencies (Pihlanto~~,~~ et al, 2008). Protein isolates and by-products were hydrolysed by alcalase, neutrase and esperase. However, all samples exhibited low radical-scavenging activity, and hydrolysis for 2 h with proteases was needed to produce an increase in the activity. For alcalase and esperase hydrolysates ultrafiltrated using a 3 kDa membrane (in comparison with 10 and 5 Kda), the scavenging capacities increased in the retentate and decreased in the permeate fraction. This works confirmed once again that bioactive peptides are low MW peptides.

Alcalase (AH), Flavourzyme (FH) and pepsin–pancreatin (PPH) hydrolysates of cowpea were fractionated by ultrafiltration (UF) (Segura Campos et al., 2010) and five fractions were prepared using four MWCOs membranes: 1, 3, 5 and 10 kDa . The antioxidant activity was not significantly different between the three hydrolysis systems, but ultrafiltration step improved their antioxidant activity, which was dependent on fraction molecular weight. Antioxidant values for UF fractions were 303.2–1457 mmol/L.mg of protein for the AH, 357.4–10 211 mmol/L.mg

of protein for the FH, and 267.1–2830.4 mmol/L.mg of protein for the PPH. The < 1 kDa peptide fraction from the FH had the highest antioxidant activity and was shown to undergo single-electron transfer reactions in a reduction assay, demonstrating its antioxidant capacity.

Rapeseed isolate was hydrolyzed by various enzymes (Alcalase, Proteinase K, Pepsin + Pancreatin (P+P), Thermolysin and Flavourzyme) and a portion of the recovered hydrolysates were subjected to UF steps, using MWCO of 1, 3, 5 and 10 kDa, for the recovery of four specific peptide fractions: <1, 1–3, 3–5, and 5–10 kDa (He et al., 2013). Overall, it was observed that the <1 kDa peptide fractions exhibited the highest DPPH radical scavenging activity with IC50 values of 0.45–0.6 mg/ml and the strongest ferric reducing antioxidant power. However, concerning chelating activity, the unfractionated hydrolysate and high MW (5–10 kDa) peptide fractions demonstrated the strongest capacity. Finally, the authors demonstrated that P + P hydrolysate was not as effective as other hydrolysates during long-term inhibition of linoleic acid oxidation (He , et al, 2013).

Red dates called Zizyphus jujuba fruits or jujubes were hydrolyzed by trypsin, papain or a combination of both enzymes (Memarpoor-Yazdi et al., 2012). The first results demonstrated that the three recovered hydrolysates exhibited antioxidant activity, however tryptic hydrolysate had the strongest activity with respective trolox equivalent antioxidant capacity, DPPH scavenging and chelating activity of 2.1 µmol TE/mg protein, 33.1 and 25.8 % at a hydrolysate concentration of 1 mg/L. Consequently, only tryptic fraction was passed through an UF membrane with a 3 kDa cut-off and the resultant ultrafiltrate was fractionated using RP-HPLC. This purification allowed the recovery of 10 fractions. Amongst these fractions, F3 and F6

demonstrated the highest activity and the most potent antioxidant peptides were identified as VGQHTR and GWLK.

Zhou et al. (2013) hydrolyzed rice proteins by three different microbial proteases (Validase, AP (alkali protease) and NP (neutral protease) produced by *Aspergillus oryzae*, *Bacillus licheniformis* and *Bacillus subtilis*, respectively). The rice protein hydrolysates were ultrafiltered sequentially under 276 kPa with MWCO membranes of 10, 3 and 1 kDa. Consequently, different peptide fractions were recovered: F1 (>10 kDa), F2 (3-10 kDa), F3 (1-3 kDa) and F4 (<1 kDa) and were tested for their ORAC, DPPH and 2,2-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) scavenging activity and their effects on meat lipid oxidation. Results demonstrated that AP-F3 and Val-F3 fractions exhibited the highest ORAC with respective values of 87.3 µmol TE/g) and 72.6 µmol TE/g. Concerning DPPH and ABTS scavenging activity, all hydrolysate fractions at 100 mg/mL scavenged 31.2 - 49.7% of DPPH and exerted remarkable ABTS scavenging activity with values ranging between 10.8 and 28.3 µmol TE equivalent/g dry weight. Finally, Val-F3 at 500 mg/g incorporated into ground beef inhibited lipid oxidation by 19% and 15% at storage day 8 and 15, respectively suggesting that rice protein hydrolysates could be used to improve shelf-life of meat products (Zhou et al., 2013).

Antioxidant activity of mungbean, (*Vigna radiata* (L.) Wilczek), also green gram, was investigated by Lapsongphon, and Yongsawatdigul (2013). In this context, mungbean meal was hydrolyzed until 24 h by three different enzymes: Virgibacillus sp. SK37 proteinases (VH), Alcalase (AH) and Neutrase (NH) and each hydrolysate was tested for ABTS scavenging

activity, metal chelating activity and ferric-reducing antioxidant power assay (FRAP). Results showed that ABTS scavenging activity increased with hydrolysis time and after 24 h of digestion, it was observed that VH and AH exhibited the strongest activity with values of about 17 mM Trolox equivalent. However, the highest chelating activity was found in AH with value of 0.4 mM EDTA. Concerning FRAP, the reducing power of all of the samples at 24 h of hydrolysis was comparable. Only VH was fractionated using UF membranes with a MWCO of 30 and 5 and three fractions were recovered: fraction I (peptides with MW >30 kDa), fraction II (5-30 kDa) and fraction III (<5 kDa). The highest specific antioxidant activity was obtained for fraction III with value of 0.16 mg Trolox equivalents /mg peptide. Finally, fraction III purification by ion exchange and gel filtration chromatography allowed the recovery of two specific peptide fractions (F37 and F42). Both consisted of four peptides but F37 fraction containing an arginine residue at their C-termini. While F37 demonstrated the highest specific antioxidant activity, F42 exhibited an important ABTS radical-scavenging activity comparable to that of 1 mM of butylated hydroxytoluene (BHT) (Lapsongphon and Yongsawatdigul 2013).

Alashi et al. (2014) hydrolyzed defatted canola meal using five food grade enzymes (Alcalase, chymotrypsin, pepsin, trypsin and pancreatin). The different hydrolysates were ultrafiltered with membrane MWCO of 1, 3, 5 and 10 kDa, for the recovery of four specific peptide fractions with MW of <1 kDa, 1-3 kDa, 3-5 kDa and 5-10 kDa. These fraction were tests for their antioxidant activities (ABTS, DPPH and superoxide radical scavenging (SRSA) activities, inhibition of linoleic acid oxidation and ORAC). Concerning ABTS, it was observed that alcalase and chymotrypsin hydrolysates were generally found to perform better than pepsin, trypsin and

pancreatin hydrolysates. However, the pancreatin <1 kDa permeate peptides fraction showed the strongest activity with an EC50 value of 10.1 lg/mL. Results of DPPH showed that pepsin <1 kDa fraction had the lowest EC_{50} values (0.18 mg/mL) compared with other peptide fractions. The same fraction exhibited the strongest antioxidant activity for SRSA with value of 51.3%. Concerning inhibition of linoleic acid oxidation, pancreatin, trypsin and Alcalase were very effective inhibitors with >50% inhibition of oxidation over the course of 7 days. Finally, the fractions that exhibited the highest ORAC values were Alcalase <1 kDa and 1-3 kDa (Alashi et al., 2014).

UF/NF

Prolastin, an elastin hydrolysate obtained by controlled proteolysis of skins from North Atlantic lean fish (Gadidae: mostly cod and pollack), was fractionated successively by UF (TMP = 30 bar) and NF (TMP = 35 bar), the UF permeate being used as the feed solution for the NF step (Picot , et al, 2010). Prolastin is composed of polypeptides with a low molecular weight (1000–5000 Da). Concentrated solutions were processed (100 g/L) at a high VRF in order to obtain four fractions with compositions as different as possible: UF retentate (enriched in peptides above 4000 Da), UF permeate (poor in peptides above 4000 Da), NF retentate (poor in peptides above 4000 Da and rich in peptides above ∼300 Da) and NF permeate (poor in peptides above ∼300 Da). According to the authors, the successive fractionation allowed the concentration of peptides of selected sizes, without, however, carrying out sharp separations, some MW classes being found in several fractions. Thus the NF retentate still contained about 22% of peptides below 300

Da. More dramatically, the UF retentate contained a large amount of peptides (93% by weight) below 4000 Da (the nominal MWCO of the membrane) even though high VRFs were reached. Peptides containing proline, aspartic acid and glutamine were concentrated in the UF and NF retentates compared to the unfractionated hydrolysate and UF permeate, respectively. The starting hydrolysate also showed a potent antioxidant and radical scavenging activity, which were not increased by UF and NF fractionation.

Very recently, Langevin et al. (2012) treated a soy protein hydrolysate sequentially by a 10 kDa UF membrane (TMP = 1.72 bar) and then the permeate adjusted at three different pHs (3, 6 and 9) was filtered by a 300-500 Da NF membrane (TMP = 50 bar). For the NF fractions, there was no significant difference on the peptide profile molecular weight according to the pH. The LC–MS analysis confirmed the MWCO of the NF membrane (300-500 Da) since the retentate fractions contained at least 50% of peptides with MW between 400 and 500 Da. Most of the peptides with a molecular weight less than 400 Da migrated in the permeate. Some peptides with molecular weights of 600 and 900 Da were also been detected in the permeate. NF molecular weight profile was not influenced by pH changes but could produce fractions with very low and specific molecular weight ranges. No significant increase were observed for the ORAC antioxidant capacity for the NF permeate and retentate and that whatever the pH. Only, the permeate from NF at pH 6 also showed a significant increase in antioxidant capacity only for the H_2O_2 degradation assay (a polarographic method) (Langevin et al., 2012). The increase in methionine in permeate pH 6 fraction could provide antioxidant properties to this fraction due to the redox properties of its sulphur group. In this work the authors compared for the first time in

the literature, a pressure-driven process (NF: 300-500 Da) with an electrically-driven process (EDFM: 10 kDa) in terms of mass flux, mass balance, molecular weight profile and protein, peptide and amino acids contents the fractions obtained both processes.

EDFM

In the comparative study of Langevin et al. (2012), it was demonstrated that NF was more efficient in terms of mass flux than EDFM when compared on a same basis (membrane area, process duration), but EDFM recovered larger range of peptide molecular weights and amount of polar amino acids. It was also demonstrated that the selectivity of EDUF was influenced by the pH values of the feed hydrolysate in comparison with NF. This confirmed that EDUF separates according to the molecular weight as demonstrated by the MW profiles and the charge of the molecule (Here controlled by the pH change). Concerning the antioxidant capacity of the fractions, the ORAC assay allowed the comparison of the antioxidant capacities of the soy peptides fractions obtained by NF and EDFM. The results showed only a significant increase in the antioxidant capacity for anionic peptide fractions recovered at pH 3 and pH 6 compared to the feed hydrolysate (Langevin et al., 2012). The antioxidant capacity of the anionic peptide fraction recovered at pH 3 could be related to the increase of glutamic acid. The increase capacity of the other anionic peptide fraction recovered at pH6 was related to the increase of some peptides with a molecular weight between 400-500 Da that may be concentrated in lysine content. The results obtained by H_2O_2 degradation confirmed some of the results obtained by ORAC for the anionic peptide fractions recovered at pH 3 and 6 showing that these fractions had

higher antioxidant capacity than the feed hydrolysate. Furthermore, the anionic peptide fraction recovered at pH 9 showed also a significant increase in H_2O_2 degradation (Langevin et al., 2012). These results, of the first study in the literature on the comparison of pressure-driven and electrically-driven processes for the fractionation of bioactive peptide fractions, showed that coupling NF and EDUF in a same process line would optimize their own separation performances and allow the production of more specific peptide fractions than alone. Indeed, as can be concluded from the works of Langevin et al. (2012) and Picot et al., (2010), the peptide size distribution observed after UF and NF fractionation demonstrated that it is misleading to characterize the fractions obtained by membrane filtration according to the MWCO of the membrane only, as is currently done in the literature.

A wide range of food matrices such as marine, plant, cereal protein hydrolysates, were studied for the concentration and the purification of antioxidant peptides by filtration processes. According to the different studies listed above, similar steps for the antioxidant peptide recovery can be summarized whatever the raw material used (Fig. 14). Thus, the first step consists in generating peptides by enzymatic hydrolysis of protein sample. In this context, several studies tested the effect of different enzymes (E) (for example trypsin (E1) or papain (E2) or pepsin (E3), etc.) while others used enzymes mixture. In both cases, the polypeptide mixture was fractionated by pressure-driven (UF or NF), integrated membrane processes (UF/NF) or electrically-driven technologies. Concerning pressure-driven processes, peptide fractions with different molecular weights were recovered according to the membrane MWCOs used. Initially, all of these peptide fractions were tested for their antioxidant activity to determine the most

promising fraction. Thus, peptide fraction which demonstrated the best antioxidant activity is purified by successive chromatographic steps until the recovery of pure peptides tested again for their antioxidant activities (ORAC, FRAP, ABTS and DDPH scavenging activities). Finally, molecular mass and amino acid sequences of the purified peptides are determined and a structure-activity relationship is occasionally given.

Concerning EDFM process, the fractionation of protein hydrolysate is performed according to the charge (anionic or cationic) and size of peptides. Consequently, the process selectivity is improved compared to pressure-driven process and allows the recovery of a very selective anionic and/or cationic peptide fraction (Fig. 15).

Conclusion

Antioxidant molecules are numerous and very different in terms of chemical structures, sizes and chemical properties according to their sources and origins. Furthermore, the fact that these molecules are generally present in complex solutions or food matrices does not facilitate their fractionation. Sometimes, the antioxidant property comes from the modification of the source such as enzymatic hydrolysis to liberate the molecules of interest.

Concerning their separation, the membrane processes must be well choosen according to the molecule physicochemical characteristics. However, in more cases, the membrane processes used do not allow the separation of pure molecule but allow the production of enriched fractions. Pressure-driven membrane processes appeared to be less selective than electrically driven

processes for a same type of molecule due to the driving force. Electricity is smoother than pressure. In addition, the selectivity of electrically-driven processes can be improved by the stacking of one or more filtration membrane: double selectivity according to the size and the charge of the molecule. However, the electrically-driven processes are limited by their recovery yield due to their high selectivity. Consequently, it is important to combine the knowledge of molecule physicochemical properties as well as process possibility to choose the adapted technology for an eco-efficient separation.

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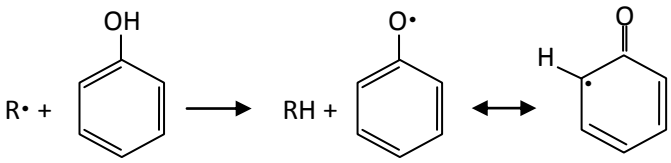


Figure 1 Reaction mechanism of phenolic antioxidant.

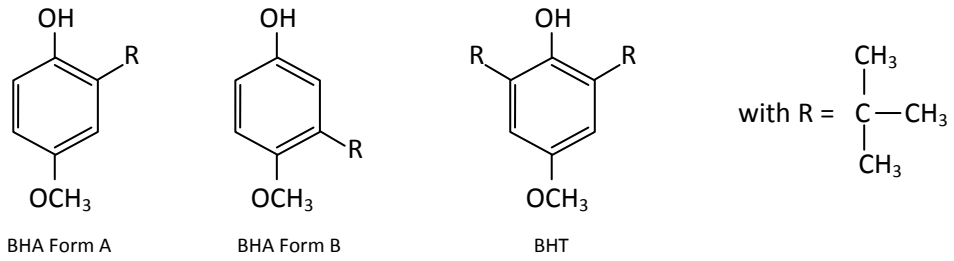
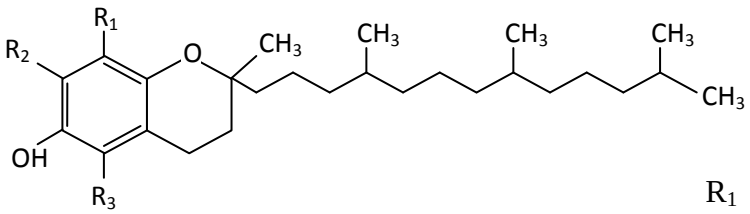


Figure 2 BHA and BHT chemical structures.



	R_1	R_2	R_3
α-tocopherol	CH_3	CH_3	CH_3
β-tocopherol	CH_3	H	CH_3
γ-tocopherol	CH_3	CH_3	H
δ-tocopherol	CH_3	H	H

Figure 3 Chemical structures of tocopherols.

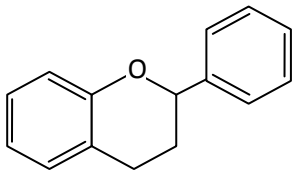
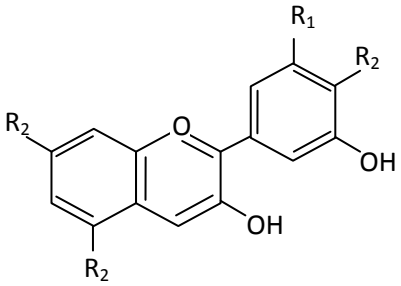


Figure 4 Basic structure of flavonoids



	R_1	R_2
cyanidin	OH	H or ose
peonidin	OCH_3	H or ose

Figure 5 Chemical structures of cyanidin, cyanidin-3-ose, peonidin and peonidin-3-ose.

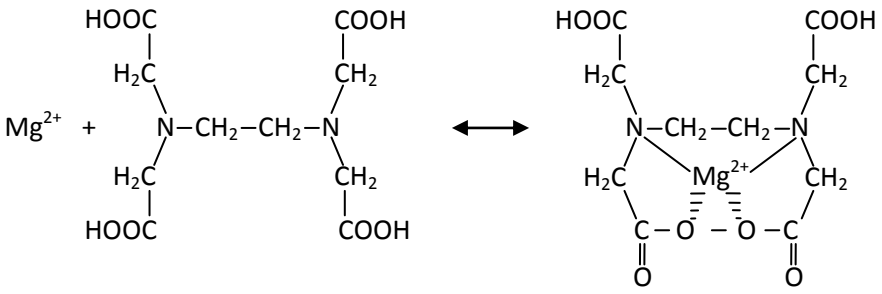


Figure 6 Complex between metal ion and EDTA.

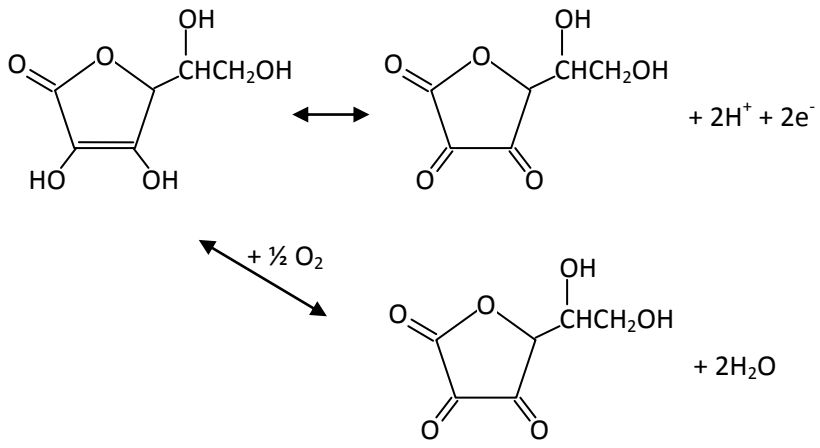


Figure 7 General oxidation mechanism of ascorbic acid and its reaction with oxygen.

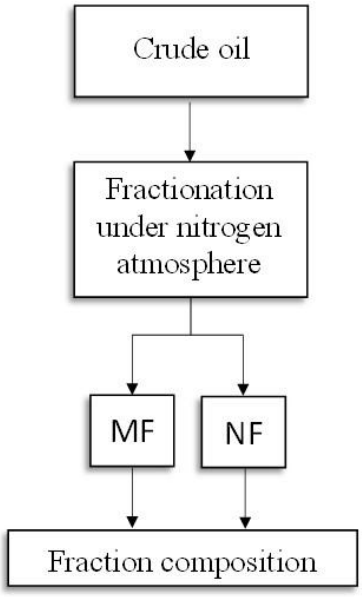


Figure 8 Successive steps for the fractionation of tocopherols and tocotrienols.

MF: microfiltration; NF: nanofiltration.

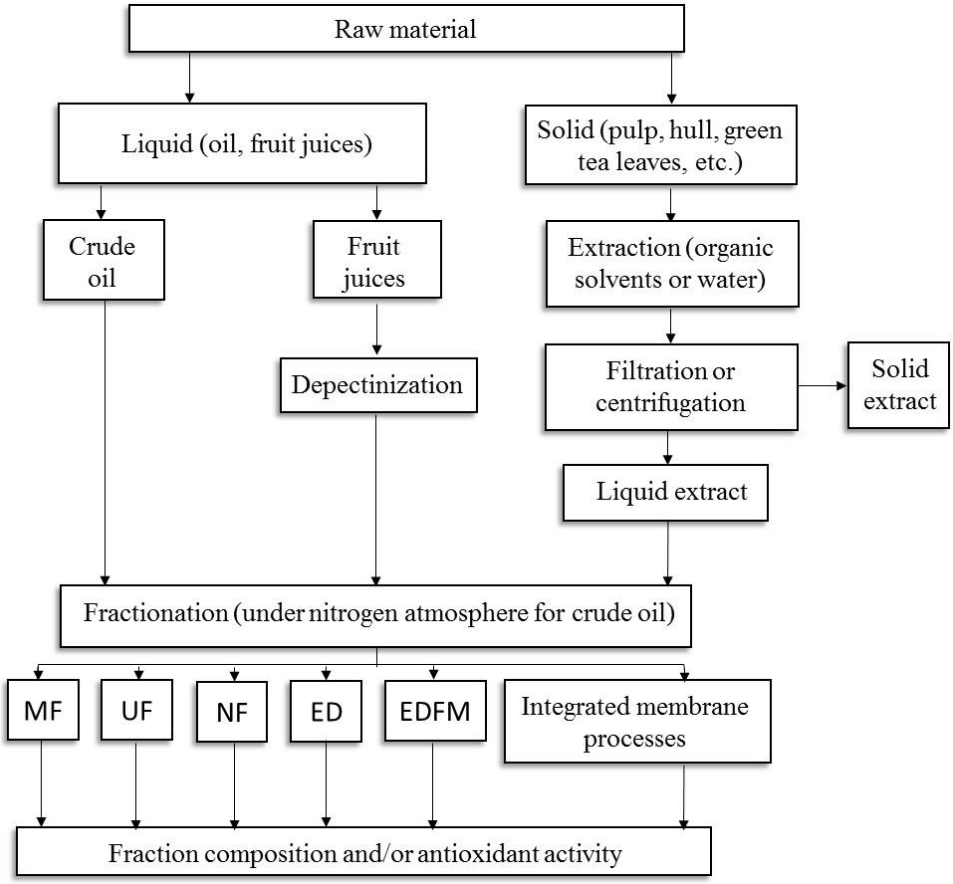


Figure 9 Successive steps for the fractionation of phenolic compounds. MF: microfiltration; UF: ultrafiltration; NF: nanofiltration; ED: electrodialysis; EDFM: electrodialysis with filtration membranes.

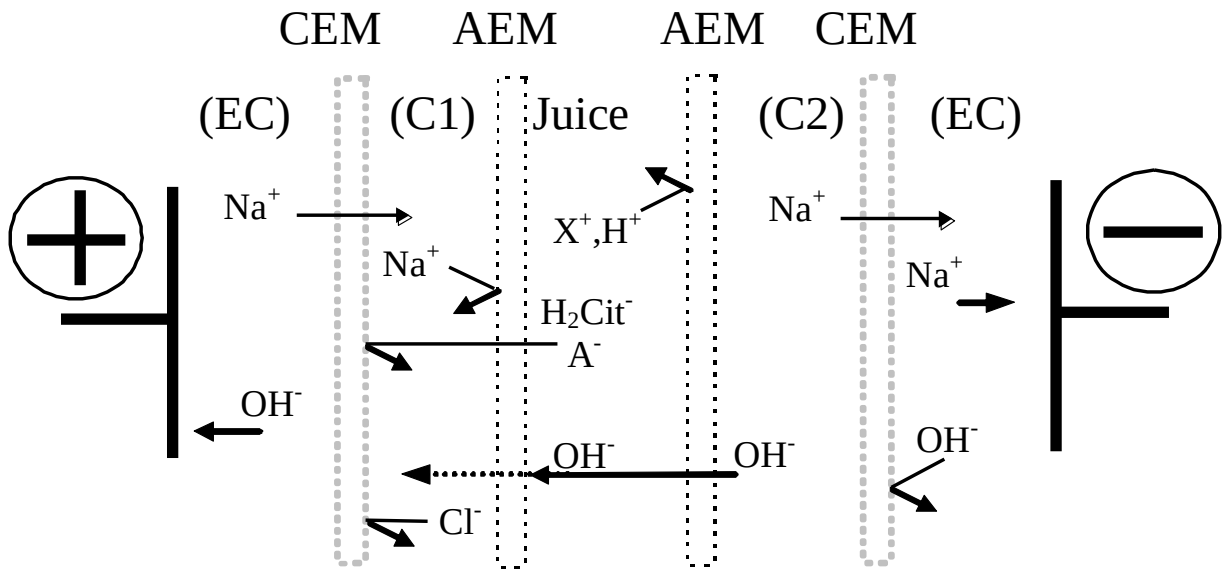


Figure 10 Configuration of electrodialysis with homopolar membranes used for juice deacidification. C1 and C2 are separated compartments while the two EC compartments are connected (ED3C configuration; AEM: anion-exchange membrane and CEM: cation-exchange membrane) (adapted from Vera Calle et al. (2003)).

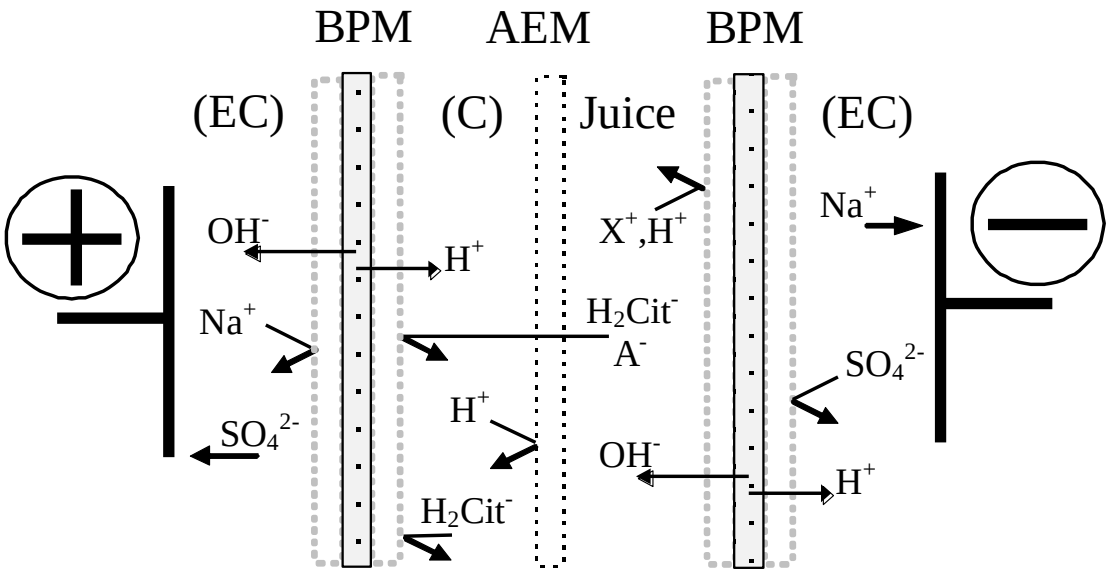


Figure 11 ED cell with bipolar membrane. (EDBM2C configuration; AEM: anion-exchange membrane and BPM: bipolar membrane) (adapted from Vera Calle et al. (2002)).

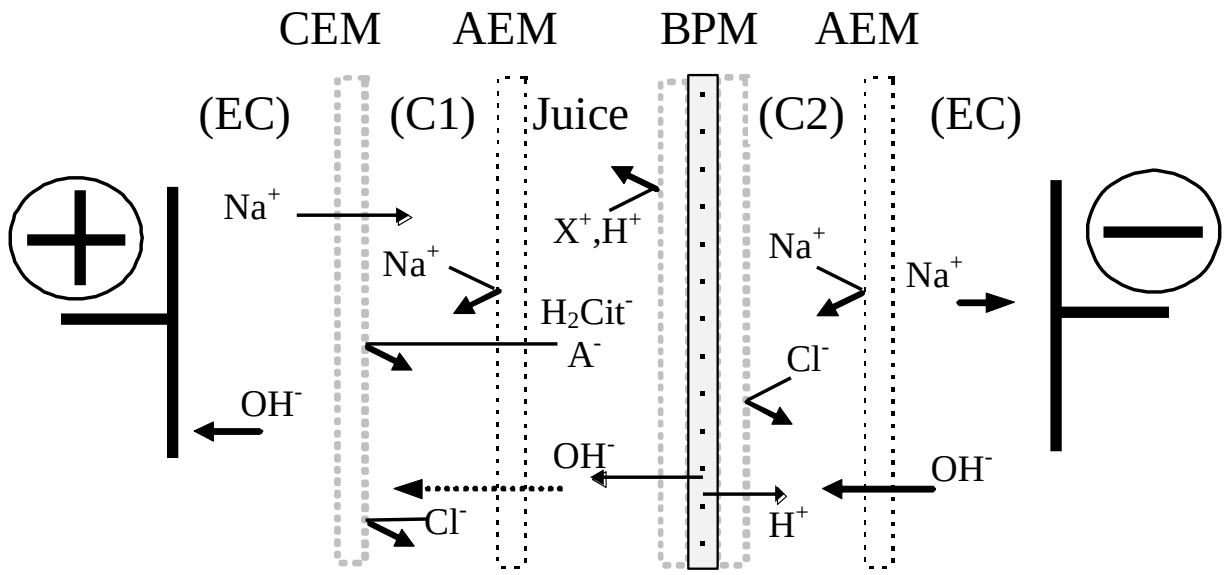


Figure 12 Modified configuration of electrodialysis with bipolar and homopolar membranes for juice deacidification. (EDBM3C configuration; BPM: bipolar membrane, AEM: anion-exchange membrane and CEM: cation-exchange membrane) (adapted from Vera et al., (2003b)).

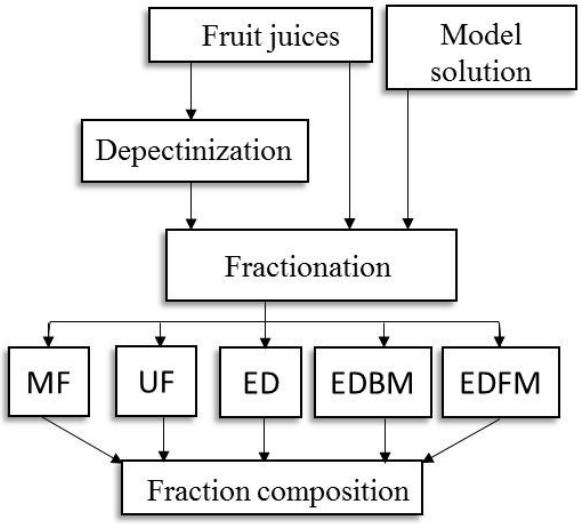


Figure 13 Successive steps for the fractionation of ascorbic, isoascorbic, folic and citric acids. ED: electrodialysis; EDBM: electrodialysis with bipolar membrane; EDFM: electrodialysis with filtration membrane.

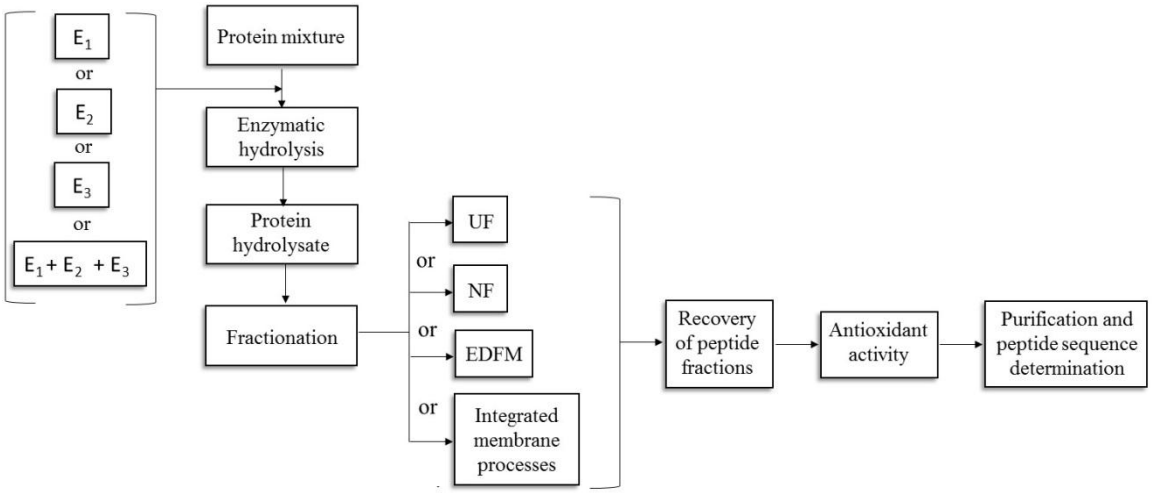


Figure 14 Successive steps for the fractionation of protein hydrolysates. E: enzyme; UF: ultrafiltration; NF: nanofiltration; EDFM: electrodialysis with filtration membrane.

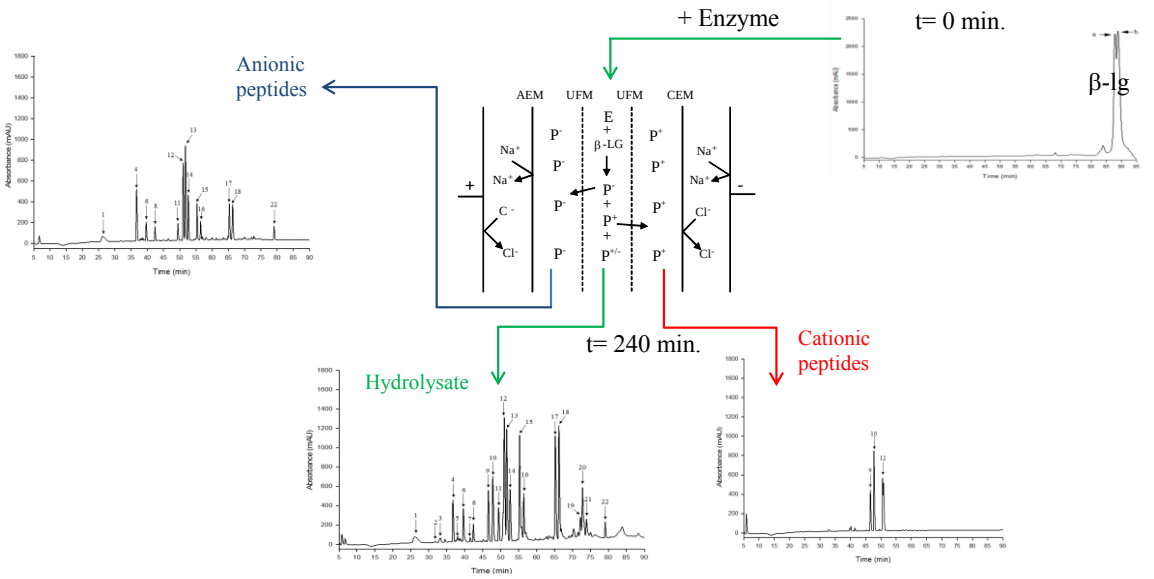


Figure 15 EDFM cell configuration used for the simultaneous hydrolysis and fractionation of peptides and respective peptides profiles in each compatment obtained at the end of the process. UFM: ultrafiltration membrane; AEM: anion-exchange membrane and CEM: cation-exchange membrane.

Table 1 Membrane processes for separation/concentration of phenols.

DRIVING FORCE	PROCESS	SOURCE	REFERENCES
PRESSURE	MICROFILTRATION	Olive oil wastewater	Russo, 2007
		Pineapple juice	Laorko et al., 2010
		Açaí pulp	Machado, 2012
	ULTRAFILTRATION	Apple juice	Wei et al., 2008
		Bergamot (Citrus bergamia Risso) juice	Conidi et al., 2011
		Citrus reticulata juice	Cassano et al., 2009
		Grape seeds	Nawaz et al., 2006
		Green tea (camellia sinensis L.)	Li et al., 2005
		Kiwifruit	Cassano et al., 2008
		Olive oil wastewater	Russo, 2007
		Pineapple juice	Laorko et al., 2010
		Propolis extract	Mello et al., 2010
		Roselle (Hibiscus sabdariffa L.)	Cissé et al., 2011
		Wine sludge	Galanakis et al., 2013
		Flaxseed hull extracts	Loginov et al., 2013
	NANOFILTRATION	Bergamot	Conidi et al., 2011
		Orange press liquor	Conidi et al., 2012
		Mate (Ilex paraguariensis)	Negrão Murakami et al., 2011 and 2013
		Roselle (hibiscus sabdariffa L.)	Cissé et al., 2011
		Sideritis ssp. L.	Tylkowski et al., 2011
		Pequi (Caryocar brasiliense Camb.)	Machado et al., 2013

	ULTRAFILTRATION + ULTRAFILTRATION	Castanea sativa	Diaz-Reinoso et al., 2011
	ULTRAFILTRATION + NANOFILTRATION	Olive mill wastewater	Cassano et al., 2013
	MICROFILTRATION + REVERSE OSMOSIS	*Justicia secunda* Vahl leaves	Koffi et al., 2013
	ULTRAFILTRATION + NANOFILTRATION + REVERSE OSMOSIS	Bergamot (Citrus bergamia Risso) juice	Conidi et al., 2011
		Olive oil wastewater	Paraskeva et al., 2007 Russo, 2007 Garcia-Castello et al., 2010
ELECTRIC FIELD	**ELECTRODIALYSIS**	Tobacco	Bazinet et al., 2005b,c
	ELECTRODIALYSIS WITH FILTRATION MEMBRANES	Cranberry juice	Bazinet et al., 2009 Bazinet et al., 2012 Husson et al., 2013
		Green tea (camellia sinensis L.)	Labbé et al., 2005

Table 2 Membrane processes for separation/concentration of antioxidant peptides from different natural sources.

DRIVING FORCE	PROCESS	SOURCE	REFERENCES
	ULTRAFILTRATION	Alfalfa leaf protein	Xie et al., 2011
		Corn protein	Zhou et al., 2012
		Rapeseed isolate	He et al., 2013
		Red dates (*Zizyphus jujuba*)	Memarpoor-Yazdi et al., 2013
		Rice protein	Zhou et al., 2013
		Mungbean (*Vigna radiata* (L.) Wilczek)	Lapsongphon and Yongsawatdigul (2013)
		Defatted canola meal	Alashi et al., 2014
		Cowpea *V. unguiculata*	Segura-Campos et al., 2010
		Egg yolk protein	Chay Pak Ting et al., 2011
		Egg white lysozyme	Rao et al., 2012
		Ostrich egg white (*Struthio camelus*)	Tanzadehpanah et al., 2012
		Hoki frame protein	Je et al., 2005a,b Jeon et al., 1999
		Bivalve mollusc (*Ruditapes philippinarum*)	Kim et al., 2013
		Blue mussel (*Mytilus edulis*)	Wang et al., 2013
		Monkfish muscle	Chi et al., 2014

PRESSURE		Potato	Pihlanto-Leppälä et al., 2008
		Purple sea urchin	Qin et al., 2011
		Shrimp processing byproduct	Zhao et al., 2011
		Soybean protein	Moure et al., 2006 Zhang et al., 2010 Roblet et al., 2012
		Whey protein	Contreras et al., 2011
	ULTRAFILTRATION + NANOFILTRATION	Prolastin	Picot et al., 2010
		Soybean protein	Langevin et al., 2012
ELECTRIC FIELD	**ELECTRODIALYSIS WITH FILTRATION MEMBRANES**	Soybean protein	Langevin et al., 2012 Roblet et al., 2012

