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Review

Nanofiltration-Assisted Concentration Processes of Phenolic Fractions and Carotenoids from Natural Food Matrices

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Abstract: In new food formulations, carotenoids and phenolic compounds are likely to be the most sought after food ingredients according to their bioactivity, nutraceutical, nutritional value, and compatibility properties once incorporated into food formulations. Such solutes are naturally present in many plant-based sources, and some portions are directly consumed when enriching food products and formulations; however, some portions, which are contained in the parts of the plant sources not considered edible, including the leaves, peel, and seeds, among other by-products, are commonly wasted. Related to this, scientists have found a new window for obtaining these bioactive molecules, but their recovery remains a challenge. To some extent, the final purification and polishing requires highly selective performance to guarantee the desired properties and concentration. In this regard, membrane technologies, such as nanofiltration (NF), represent an alternative, owing to their highly selective properties when separating low-molecular-weight compounds. NF becomes immediately suitable when the pretreated extracts are subjected to further efficient concentration, fractionation, and polishing of phenolic fractions and carotenoids. The separation efficiency (usually higher than 97%) of NF technology is high according to the low pore size of NF membranes, but the low temperature in process separation also contributes to the separation of thermolabile compounds. Therefore, this paper reviews the ongoing cases of studies reporting the successful separation and polishing of phenolic fractions and carotenoids from distinct sources. In particular, we have focused our attention on the main interactions during the separation process and the drawbacks and advantages of using membranes for such a case study.

Keywords: nanofiltration; universal recovery process; phenolic fractions; food systems; carotenoids

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1. Introduction

Membrane technologies represent an economically feasible method for the recovery and polishing of bioactive molecules sought by pharmaceutical and food industries. Membrane technologies have become of interest for scientists since they are low-energy-expenditure processes [1], have no need of additional phases for the separation of the target solutes, require minimal steps of processing, and do not use temperature gradients for efficiency separation [2,3]. This latter characteristic makes them more attractive for obtaining highly thermolabile compounds since they demand the absence of temperature to not compromise their stability and bioactivity. As for micro (MF)-, ultra (UF)-, and nanofiltration (NF), the transmembrane pressure is identified as the main driving force for carrying out the process. These processes differ from each other in the average pore size of the membrane used [4]. In the domain of bioactive molecule retrieval, the preeminence of MF has become conspicuously apparent as the most widely employed approach for the preliminary treatment of bulk solutions, primarily targeting the elimination of particulate matter and macromolecular constituents. Conversely, in the pursuit of recuperating high-molecular-weight entities, such as carbohydrates, proteins, pectins, and abundant phenolic fraction with considerable molecular mass, UF prevails as the commonly favored

method [5]. As the finest pore-sized membranes, ranging from 2 to 0.5 nm (350 and 1000 Da), NF is the most effective process for separating low-molecular-weight compounds [6], such as phenolic fractions and carotenoids. To some extent, the membrane pore size determines the separation efficiency of the processing depending on the molecular weight of the solutes; however, specific interactions (such as Coulombic, polar, and hydrophobic interactions), operating parameters (pressure, pH, temperature, etc.), and phenomena (such as membrane fouling) greatly influence the overall performance of the NF process during the separation task [7–9]. Therefore, apart from reviewing the ultimate recovery cases of phenolic compounds and carotenoids from distinct food systems, we also examine the main parameters and interactions occurring during the recovery process and contributing to a superior recovery percentage. Finally, we provide the main perspectives to be studied by scientists to make NF completely attractive in the recovery of food ingredients.

2. Ongoing Progress in Recovery of Phenolic Fractions from Food Systems via NF

2.1. Flavonoids

Flavonoids (FVs), which are solutes of low molecular weight, chemically present 15 carbon atoms that are organized in a C_6–C_3–C_6 configuration. The chemical structure of FVs presents two either different or similar aromatic rings depending on the FV type, which are linked by a three-carbon bridge, commonly in a heterocyclic ring configuration. Due to this change in such a heterocyclic ring configuration, it is possible to obtain six different subclasses of FVs, as represented in Figure 1. In general, flavonols, flavanols, isoflavones, flavones, flavanones, and anthocyanidins are typically derived from this FV pattern modification [10–12].

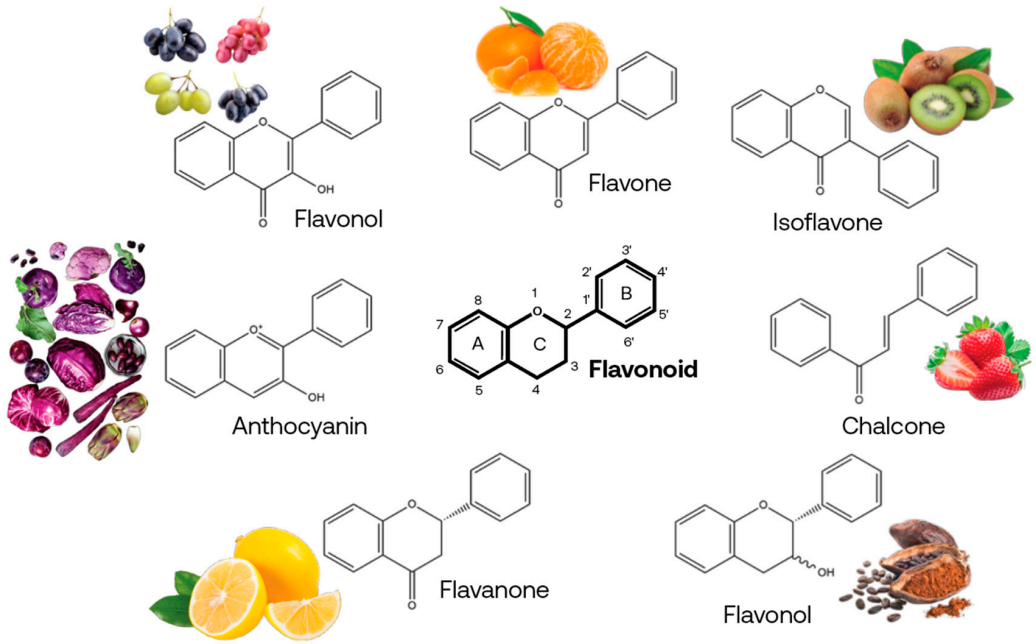

Figure 1. Typical flavonoid chemical structure and its derived variants.

According to the USDA database, several variants can be found in each subclass, as follows:

- Flavonols: quercetin, kaempferol, myricetin, isorhamnetin;
- Flavan-3-ols: catechins, epicatechins, epicatechin3-gallate, epigallocatechin, epigallocatechin 3-gallate, gallocatechin, theaflavin, theaflavin 3-3′-digallate, theaflavin 3′-gallate, theaflavin 3-gallate, thearubigins;
- Flavones: apigenin, luteolin;
- Flavanones: hesperetin, naringenin, eriodictyol;
- Anthocyanidins: cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin.

The concentrated phenolic fractions and carotenoids derived from advanced separation methodologies, such as NF or UF, manifest an expansive array of practical applications and prospective advantages across diverse industries, notably within the domain of food formulations and allied sectors [13].

In the intricate landscape of food science and technology, these concentrated bioactive compounds assume a pivotal role in elevating both the nutritional composition and sensorial attributes of comestible products. Phenolic compounds, distinguished for their discernible antioxidant prowess, efficaciously attenuate oxidative processes, thereby conferring prolonged shelf life upon perishable edibles. The incorporation of concentrated phenolic fractions into formulations not only begets augmented food preservation but also accords with the burgeoning consumer proclivity toward natural antioxidants as a judicious alternative to synthetic additives.

Simultaneously, carotenoids, as colorant agents, confer vibrant hues to comestibles, concurrently endowing them with essential provitamin A activity. The judicious concentration of carotenoids through nanofiltration enables meticulous control over color attributes, facilitating the fabrication of visually captivating foods without the reliance on synthetic colorants. Furthermore, the antioxidant potential inherent in specific carotenoids contributes substantively to the overall oxidative stability of lipid-laden food formulations [14].

Transcending the gastronomic milieu, these concentrated bioactive compounds have carved out niches in the pharmaceutical and cosmetic spheres. Phenolic fractions, exhibiting anti-inflammatory, antimicrobial, and potentially anticancer attributes, have emerged as pivotal constituents in the synthesis of functional foods or pharmaceutical formulations. Meanwhile, carotenoids, acknowledged for their cutaneous health benefits and free-radical-quenching capabilities, are habitually incorporated into cosmetic formulations for skincare products [14].

In the agricultural field, the utility of concentrated phenolic fractions extends beyond mere food-processing applications. These compounds, evincing efficacy as plant growth regulators and elicitors, may provide heightened crop yields and resilience. In parallel, carotenoids, owing to their involvement in photosynthetic and stress-responsive mechanisms, may offer amplified plant vitality and productivity [14].

Furthermore, the ramifications of these concentrated bioactive compounds extend to the environmental field. The chelating properties inherent in phenolic compounds may be harnessed for water treatment, efficaciously mitigating the deleterious impacts of heavy metal contamination. In a parallel vein, the light-absorbing proclivities of carotenoids may be instrumentalized in solar energy applications, delineating an intersection between biological entities and sustainable technological pursuits [15].

The judicious application of concentrated phenolic fractions and carotenoids, procured through sophisticated separation methodologies such as nanofiltration, is poised to orchestrate a transformative paradigm shift across diverse industries. From fortifying the nutritional fabric of food commodities with natural antioxidants to contributing substantively to pharmaceuticals, cosmetics, agriculture, and environmental stewardship, the multifaceted applications underscore the profound and far-reaching influence of these bioactive compounds on human well-being, aesthetic considerations, and sustainable practices within various scientific domains.

In recent investigations, it has been confirmed that there are multiple health-promoting benefits from the consumption of FVs in the diet [16]; for example, they significantly reduce the risk of developing chronic illnesses including cardiovascular diseases, diabetes (mostly type II), and distinct types of cancers [17]. Given their related bioactive properties, food formulations and products may possibly be enriched with FVs to increase the nutritional value of the final products. Despite their availability in most fruits and vegetables, the extraction of FVs represents a challenge due to the chemical complexity of the natural source. Apart from this, most of the traditional extraction techniques may use the application of temperature gradients for high extraction yields. This represents a constraint due to the high thermal degradability of FVs along with the long extraction time [18]. In this regard,

scientists strongly seek extraction methods exhibiting properties of low reactivity and thermal degradability of FVs to ensure both acceptable yield and bioactivity of the solute. Here, membrane technologies, especially driven by a pressure gradient, are alternatives for such extraction tasks since they do not use any temperature gradient. With high selectivity, NF can efficiently concentrate distinct types of FVs from distinctive natural products and their byproducts, as listed in Table 1. The capability (in terms of rejection towards FV rejection) of different commercial NF membranes varied from 52 up to 99% depending on the type of feed source and the properties of the membrane, including MWCO, membrane structure, and membrane material.

Table 1. Concentration processes of FVs from natural products and by-products using different commercial NF membranes.

Source	Membrane Specifications			Best Performing Membrane	Flavonoid Rejection	Reference
	Commercial Membrane	Polymer Material	MWCO (Da)			
Grape by-products	AFC40	Polyamide	60% $CaCl_2$	Integrated membrane system	100%	[19]
	PU608	Polysulphone	8000			
	PU120	Polysulphone	20,000			
	FP200	Polyvinilidene fluoride	200,000			
	Nanomax 95	Polyamide/Polysulphone	250	Inside Céram	52%	[20]
	Nanomax 50	Polyamide/Polysulphone	350			
	DL2540	Thin film	150–300			
	GE2540	Thin film	1000			
	Inside Céram	Titania	1000			
	CA400-22	Cellulose acetate	n/a	NF270	93.8%	[21]
	CA400-26	Cellulose acetate				
	CA400-28	Cellulose acetate				
	NF270	Polypiperazine				
	ETNA01PP	Fluoropolymer				
	NF270	Polypiperazine	200–300	Integrated membrane system	ACN: 100% TPC: >90%	[22]
	ETNA01PP	Fluoropolymer	1000			
	ETNA10PP	Fluoropolymer	10,000			
	M-U2540	Polyacrylonitrile	20,000	HYDRACoRe 70pHT	91.9%	[23]
	HYDRACoRe 70pHT	Sulfonated polyethersulfone	720			
	NF270	Polypiperazine	97% $MgSO_4$			
	NF90	Polyamide	97% $MgSO_4$			
	ESP04	Modified polyethersulfone	4000			
	HFW1000	Modified polyethersulfone	1000			
	NF270	Polypiperazine	300	NF270	>90%	[24]
	ETNA01PP	Fluoropolymer	1000			

n/a: Not Applicable.

The intricate process encompassing the recovery, purification, and concentration of phenolic compounds from produce sources through a multifaceted integration of membrane operations is elucidated in Figure 2. In this latter scheme, a systematic framework is meticulously designed to exploit the inherent selectivity and permeability of membranes in separating, purifying, and concentrating these bioactive constituents from the complex matrices of vegetable extracts.

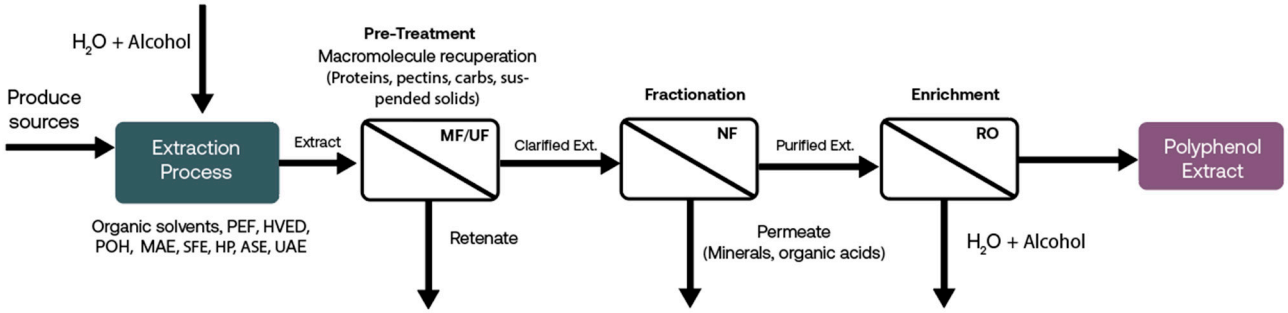

Figure 2. Procedural framework for the concentration of phenolic compounds extracted from produce sources by membrane processing.

Solvent extraction is a widely used and effective method for the extraction of phenolic fractions and carotenoids from natural sources. The choice of solvent depends on the specific characteristics of the target compounds and the source material. The use of green solvents and the optimization of extraction conditions can improve the efficiency and sustainability of the extraction process.

A review article published by Saini et al. [25] described the utilization of non-polar solvents, such as hexane, as underscored for the extraction of carotenoids owing to their hydrophobic nature. Additionally, the article explored the efficacy of polar solvents like ethanol and methanol in extracting phenolic compounds. Conversely, a study featured by Ricarte et al. [26] investigated the application of enzyme-assisted extraction alongside green solvents such as ethyl lactate and limonene for extracting carotenoids and phenolic compounds from sunflower wastes, revealing enhanced extraction efficiency with green solvents. Similarly, the utilization of green solvents includes vegetable oils, supercritical fluids, deep eutectic solvents, and ionic liquids for extracting carotenoids from fruit and vegetable by-products [27], emphasizing the necessity of safe solvents and product recovery for large-scale applications. On a different note, a study made by Wang et al. [28] scrutinized the extraction and recovery of bioactive soluble phenolic compounds from grape pomace, highlighting methanol as the most efficacious solvent. Furthermore, a review article published by Garcia-Salas et al. [29] analyzed various solvent systems for extracting phenolic compounds from fruit and vegetable samples, elucidating the advantages and drawbacks of methods such as liquid–liquid extraction, supercritical fluid extraction, solid-phase extraction, microwave-assisted extraction, and ultrasound-assisted extraction.

Pre-treatment procedures preceding membrane filtration influence the operational efficiency and long-term operation of membrane-based processes. These pre-treatment procedures, including coagulation, flocculation, sedimentation, and pre-filtration, are meticulously designed to address fouling mechanisms, enhance permeate quality, and optimize hydraulic performance. By targeting the removal of particulate matter, colloids, and macromolecules, pre-treatment mitigates fouling propensity, thereby extending membrane lifespan and reducing the frequency of maintenance. Furthermore, pre-treatment strategies enable the control of foulant concentration, regulating organic and inorganic species that could compromise membrane performance. This proactive approach not only preserves permeate quality but also minimizes energy consumption by optimizing pressure differentials across the membrane surface.

Cassano et al. [30] conducted an assessment of an integrated membrane process employing UF and nanofiltration NF membranes for the recovery of phenolic compounds from olive mill wastewaters (OMWs). Their study revealed that UF pre-treatment resulted in diminished permeate flux decay and elevated steady-state permeate flux values during subsequent UF steps utilizing a composite fluoro polymer membrane with a molecular weight cutoff of 1 kDa (Etna 01PP, Alfa Laval, Nakskov, Denmark). Furthermore, a prior investigation demonstrated that pre-treating raw OMWs with a ceramic MF membrane before NF application yielded substantial reductions in total suspended solids (TSS) and

total organic carbon (TOC) by 91% and 26%, respectively. Within the MF permeate, 78% of phenolic compounds were successfully recovered and separated from suspended solids and partially from other organic constituents. The MF membrane exhibited varied rejection rates towards different low-molecular-weight phenolic compounds, ranging from 7.2% (protocatechuic acid) to 27.7% (oleuropein) [31].

The necessity for stereochemistry analysis arises from the intricate interplay between the distinctive structural configurations of polyphenols or carotenoids and the nuanced physicochemical properties inherent to nanofiltration or ultrafiltration membranes. Each chemical entity exhibits a complex stereochemical landscape, necessitating meticulous examination to discern enantiomeric and diastereomeric arrangements. This analytical endeavor is indispensable for comprehending the intricate molecular recognition phenomena and transport dynamics occurring at the interface of membranes [32]. The molecular configurations and functional moieties inherent in phenolic fractions and carotenoids exert a profound influence on their behavior within polymeric membrane matrices, influencing interactions with porous structures and membrane additives in membrane processes. Carotenoids, characterized by repetitive isoprene units interspersed with conjugated double bonds, demonstrate precipitating structural alterations including ring cycling, double bond migration, and oxygen molecule addition [33]. Conversely, phenolic compounds present a diverse array of structures featuring hydroxyl groups, conferring antioxidant properties and fostering interactions with membrane substrates [34]. The presence of aromatic rings and aliphatic chains imparts a hydrophobic character to these micromolecules, augmenting their molecular volume. However, an escalation in the abundance of hydroxyl and carboxylic groups, alongside an acidic pH characteristic of the feed samples, engenders intermolecular negative polarity. Consequently, solutes engender water molecule attraction, resulting in increased volumetric dimensions of target molecules and impeding their permeation through membrane pores, thus engendering the "polarity resistance" phenomenon [35,36].

Moreover, the selection of NF or UF membranes necessitates a comprehensive understanding of their pore characteristics, surface charge density, and hydrophobicity gradients, all of which collectively dictate compatibility or reactivity thresholds with phytochemical constituents. Through the synergistic integration of spectroscopic techniques and computational modeling, this holistic approach not only elucidates the stereochemical information but also serves to inform the design and optimization of the membrane processes tailored for the efficient separation, purification, and valorization of polyphenolic and carotenoid compounds across diverse industrial and biomedical sectors. When selecting polymeric membrane materials, careful consideration must be given to selecting membranes that exhibit chemical stability and compatibility with the functional groups present in the target compounds. Furthermore, the porous architecture of the membrane and the incorporation of additives, such as polyhydroxy butyric acid and polyethylene glycol, can significantly impact the permeation rates and the efficiency of separation and the retention of phenolic fractions and carotenoids. Hence, the choice of membrane materials, pore size, and surface modifications should be meticulously tailored to the distinct characteristics of the phenolic fractions and carotenoids, to achieve optimal separation and retention within integrated membrane processes. In the context of polysulphone membranes, the sequential arrangement of aromatic and aliphatic units confers upon the polymer its hydrophobic profile, thereby effectively repelling water and hydrophilic compounds [37,38]. These units are intricately linked with oxygen (aryl-O-alkyl) and sulfur dioxide (aryl-SO_2-alkyl) molecules, intermittently introducing hydrophilic properties to the membrane surface through the formation of hydrogen bonds. Despite the predominantly hydrophobic nature of polysulphone, it remains vulnerable to concentration polarization and fouling, primarily attributed to the deposition of organic matter [39].

The pronounced retention of micromolecules within large membrane pores is ascribed to their inherent polarity. Phenolic compounds, for example, exhibit both nonpolar and polar moieties within their molecular structures. The hydroethanolic solvent utilized in

specific applications serves to shield these dual sides by furnishing ethanol molecules to the nonpolar side and water molecules to the polar side, thereby enhancing their solubility and flexibility. This phenomenon not only augments their solubility but also potentially contributes to the preservation of their antioxidant properties throughout storage periods [40]. Conversely, this inherent flexibility may facilitate the traversal of phenolic compounds through membrane pores, enabling them to navigate and adhere within the narrower porous regions of polysulphone membranes. The c molecular weight of the solutes, as well as their polar and non-polar regions, along with the membrane's polarity and molecular weight cutoff (MWCO), results in a decreased retention of phenolic compounds observed with a more polar membrane, such as cellulose acetate with a 30 kDa cutoff [41].

The MWCO of the membrane represents merely one aspect among several criteria to consider. Notably, the asymmetrical fabrication of membrane pores does not consistently correlate with a narrow MWCO range. Furthermore, various phenomena, such as concentration polarization, membrane fouling, and Coulombic and hydrophobic interactions, contribute significantly to phenolic retention [42,43]. The impact of these phenomena diminishes when considering the solubility of solutes and the hydrophobicity of the membrane surface [42]. Consequently, the MWCO serves as a relative rather than absolute barrier for the segregation of macro- and micro-molecules. Additionally, within bioresource matrices, larger and smaller functional molecules tend to aggregate into clusters; for instance, phenols may non-covalently bind to dietary fibers or proteins [35]. A reduction in the MWCO of the membrane is also evident during polarization concentration and fouling issues. Furthermore, polyphenols exhibit a propensity to interact and bind non-covalently to proteins and polysaccharides. This implies that low-molecular-weight polyphenols may concentrate in the retentate, aligning with the structural characteristics of higher-molecular-weight polyphenols [44].

The efficacy of membrane performance hinges upon a multitude of factors meticulously controlled to not only optimize permeate flux but also enhance selectivity towards target compounds [45–47]. Among these factors, transmembrane pressure (TMP) assumes a pivotal role, exerting a direct influence on membrane fouling, which can manifest as reversible or irreversible. Notably, increasing TMP results in a proportional augmentation of permeate flux until reaching the critical transmembrane pressure, where this linear correlation becomes attenuated. This critical threshold signifies the apex of the flux attainable under specific operational parameters, beyond which further increments in TMP yield no commensurate enhancement in flux [48]. Consequently, for the seamless continuity of processes, TMP must be judiciously set below the critical value, thus operating within a non-critical zone characterized by minimal fouling effects [6].

The rejection of phenolic compounds typically exhibits an upward trend with the elevation of TMP, a phenomenon well-documented in the literature [20,49]. This phenomenon finds explanation within the framework of the film layer theory, positing the formation of a thin layer adjacent to the membrane surface characterized by a specific thickness, wherein the concentration gradient diminishes from the surface towards the bulk solution. With escalating TMP values, concentration polarization and fouling phenomena become more pronounced, culminating in the deposition of an additional selective layer atop the membrane surface, thereby increasing the retention coefficient [6]. In the treatment of ultrafiltered OMWs using NF membranes, the fouling effects are observed with the increase in operating pressure [50]. Typically, elevated temperatures yield increased flux rates across both the pressure-controlled region (below the critical TMP) and the mass transfer-controlled region (above the critical TMP). This temperature-dependent effect stems from the reduction in feed viscosity, thereby necessitating diminished pumping energy and horsepower [51]. Nevertheless, in applications where membrane processes are employed for phenolic compound recovery, operational temperatures should ideally be maintained at lower levels to mitigate the risk of compromising the bioactivity of the compounds.

As can be seen in Table 1, agro-food by-products, such as blueberry pomace, pomegranate wastes, citrus, grape and artichoke by-products, are likely to be the most investigated

sources for obtaining these bioactive molecules. For instance, grape (*Vitis vinifera* L.) is likely to be one of the principal fruit crops worldwide [51,52]. It is consumed raw or processed into other products. It is known that around 75% of the worldwide grape production is commercialized and used in the wine-making industry. In particular, grape pomace, which represents ca. 25% of the entire grape weight, is a large by-product from the wine-making industry. This by-product is formed of different grape parts such as skin, stalks, and seeds [53]. To some extent, these wine-making by-products contain different classes of flavonoids, including monomeric flavan-3-ols and oligomeric proanthocyanidins [54]. In a strategic fractionation of this by-product, Santamaria et al. [19] first reported the fractionation of defatted milled grape seeds to obtain a final extract enriched in phenolic fractions via AFC40 NF membrane, which successfully eliminated low-molecular-weight acids and aldehydes. After this, the extract was further filtered using PU608 and PU120 UF membranes. The resulting UF retentate was subsequently filtered with FP200 MF membrane to successfully recover pure oligomeric proanthocyanidin extract. Strategically, a PU608 UF membrane was implemented to fractionate the proanthocyanidins into two fractions: (i) an extract containing dimers and trimers, and (ii) an extract containing oligomeric proanthocyanidins [19]. A recovery of 100% of the total FVs can likely be obtained. Applying different membranes, Diaz-Reinoso et al. [55] experimented with the recovery of phenolic fractions from distilled fermented grape pomace in water solutions. Here, the maximum phenolic fraction recovery was observed as 52% using distinct commercial UF and NF membranes, as found in Table 1. Importantly, all tested membranes were used in a single-stage unit observing the molecular sieving as the main parameter for the efficient separation. However, several membranes configurated in sequence reveal a greater recovery rate, as reported by Santamaria et al. [19].

By developing lab-made membranes based on cellulose acetate membranes, namely CA400-22, CA400-26, and CA400-28, Giacobbo et al. [56] compared their performance in phenolic fraction recovery from winery effluents with two commercial nanofiltration membranes (NF270 and ETNA01PP). In particular, the commercial NF270 membrane exhibited the maximum rate for phenolic fractions as high as 93.8%, while the other commercial ETNA01PP offered a poor rejection of ca. 27% [56]. As for lab-made membranes, they displayed a recovery ranging from 40 to 70%. The low recovery rate could be attributed to the hydrophilic profile that CA membranes tend to display. The same authors reported the UF membrane (MWCO: 10 kDa) as the suitable one for splitting the phenolic fractions from polysaccharides, while they also found that an NF membrane was able to retain almost all anthocyanins with 90% of the total phenolic compounds [57]. In a subsequent study by the same researchers [58], it was observed that for both commercial membranes, the ETNA01PP and NF270 membranes, the concentration polarization phenomenon increases by increasing the transmembrane pressure (at low crossflow velocities) [58].

Organic acids (such as tartaric acid) and phenolic fractions have been acquired from winery waste lees [59]. In this development, among the different NF membranes, sulfonated polyethersulfone, labelled as 70pHT, revealed interesting recovery performance as high as 91.9% while exhibiting minimal fouling [59]. By using the proposed scheme recovery process (see Figure 3), the high phenolic fraction recovery has been ascribed to some extent to the possible interaction with polysaccharides, which improved the phenolic solubility in water. Interestingly, the suggested recovery process involves wine lees, as recovered without any drying process, which can be mixed with acidified water [59]. After a sedimentation process, by incorporating cationic resins, the potassium bitartrate is dissolved, yielding tartaric acid, while phenolic fractions embedded/entrapped in the wine lees matrix are also dissolved in the acidified water. After this, the obtained supernatant can be fractionated by an NF membrane, ranging from 720 to 1000 kDa. In general, the proposed membrane stage can distinguish tartaric acid from phenolic fractions, in which the rejection rates will depend on the membrane cutoff and the final chemical composition of the treated bulk [60].

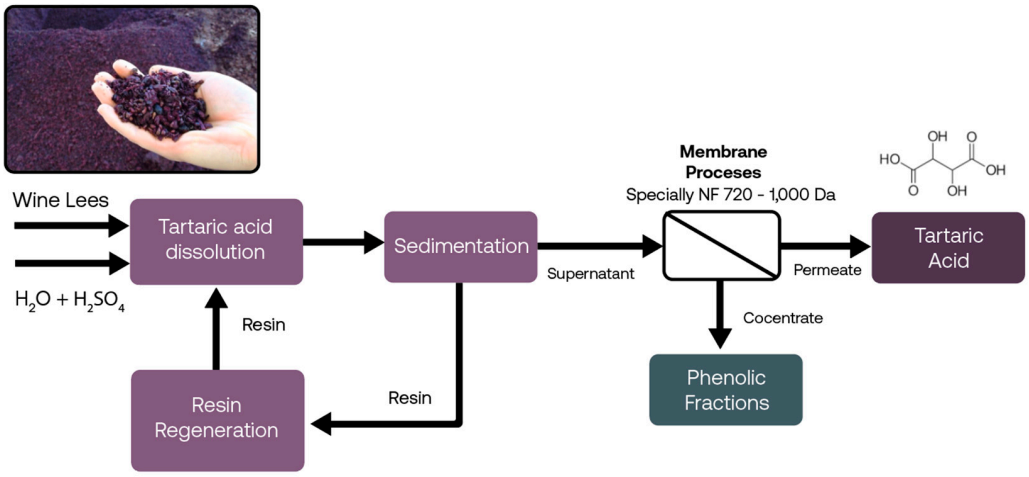

Figure 3. Suggested process diagram for the recovery of tartaric acid and phenolic fractions from wine lees using NF steps. Inspired by Kontogiannopoulos et al. [59].

Investigating nine different NF membranes with a cutoff between 150 and 1000 Da, Yammine et al. [61] evaluated the efficient separation of phenolic solutes from grape pomace extracts. The membranes, exhibiting a cutoff between 500 and 1000 Da, were efficient enough to retain polymeric proanthocyanidins, while membranes presenting 300–600 Da were capable of fractionating monomeric compounds. To recover polymeric flavan-3-ols (over 370 g/mol molecular weight), the rejection values varied from 59 to 100%, representing exceptional recovery efficiency, while for molecules smaller, like catechin (ca. 290 g/mol), the rejection efficiency behaved randomly from 23 up to 99.4%. When implementing membranes with a narrower cutoff, e.g., between 150 and 400 Da, the rejection efficiency for flavonoids and anthocyanins was reported to be more stable, ca. 95% according to the experiments of Yammine et al. [61].

It is likely that grape by-products are the most investigated wastes due to their high content of phenolic compounds, as shown in Table 1; however, citrus fruits, berries, and pomegranate are among important fruits with high content of flavonoids and they are also investigated to obtain phenolic fractions. As an example, Cassano et al. [62] initially examined the separation and later the concentration of flavonoids contained in orange press liquor produced after orange peel processing. In this work, four distinct NF membranes in spiral-wound configuration were used with cutoff from 250 to 1000 Da. Interestingly, the authors, as experts in the field, also paid attention to different membrane materials and their effect on the resulting physicochemical and separation properties of the membranes. Here, the authors used diverse polymer materials including polyamide, polypiperazine amide, and polyethersulfone. The findings determined that the highly hydrophobic NFPES10 membrane based on polyethersulfone exhibited the highest rejections for anthocyanins, ca. 89%, while for flavonoids, it was about 70% [62]. In this regard, it is worth mentioning that the membrane hydrophobicity exerts a noticeable effect during the separation process. This is the case of polysulfone, which exhibits a hydrophobic profile and thus repels water molecules and all related soluble solutes with a hydrophilic nature. The hydrophobicity of this polymer is thanks to its chemical structure presenting sequential aromatic and aliphatic units, and hence showing this characteristic [63].

The same research group looked at citrus juice from bergamot (*Citrus bergamia Risso*) as a source of flavonoids. In this development, Conidi et al. [64] implemented a sequence of membrane stages based on UF and NF. First, bergamot citrus extract was ultrafiltrated to eliminate the suspended solids. The clarified extract was then processed by distinct UF and NF membrane modules with different membrane cutoff; this was proposed to analyze the phenolic fraction rejection together with sugars and organic acids. In particular, the NF membranes, exhibiting a cutoff of 450 Da, displayed the highest phenolic fraction rejection, which varied from 91 to 99% [64]. An important finding documented by the

authors relies on the fouling phenomenon, which was noted to be more severe when the pH of the solution was increased from 2.8 to 8.5. To some extent, such an increase in pH brings the precipitation of the phenolic compounds, which consequently promotes the retention of other molecules [65]. By experimenting with distinct commercial NF membranes, the authors also recovered flavonoid fractions with recovery rates between 88.4 and 90.1% [66].

Aronia, which is a typical violet-black fruit berry from North America, presents significant content of anthocyanins [67]. By processing this natural juice, an almost complete recovery (ca. 99%) has been reported by Gilewicz-Łukasik et al. [68] using an NF membrane; however, the great concentration of the bioactive ingredients was also assisted by using Na_2SO_3, which is recognized as an efficient extractant. In a more recent development, Arend et al. [69] utilized subsequent MF and NF stages to concentrate anthocyanin-containing strawberry (Fragaria X ananassa Duch) juice. Here, pelargonidin 3-O-glycoside was most found and retained at ca. 95%. According to the authors, a possible interaction between this phenolic solute and other compounds (such as low-molecular-weight polysaccharides) may generate chemical bonds and the Van der Waals force between the complex compounds and membrane material, contributing to such rejection rates [69].

Blackberry (*Rubus adenotrichos Schltdl.*) extract, replete with a profusion of anthocyanins and ellagitannins, underwent concentration employing an assortment of NF membranes. Notably, each of the examined membranes exhibited unparalleled efficacy in retaining 100% of the aggregate ellagitannin content, whereas, at a volumetric reduction ratio of 1, an astonishing retention exceeding 94% was achieved for the total anthocyanin fraction. Intrinsically, the NF270 membrane, subjected to an operating pressure of 3 MPa, emerged as the paramount contender, showcasing the most elevated potential for the concentration of blackberry polyphenolic compounds [69]. Blueberry (*Vaccinium corymbosum*) pomace, as the residual solid of juice processing, is a fruit by-product containing a high loading of both monomeric and polymeric anthocyanins. Avram et al. [70] deployed a dual array of NF membranes, namely NF270 and NF245, as adept tools for the extraction of anthocyanins, flavonols, and an array of other phenolic compounds from blueberry pomace. Both NF270 and NF245 membranes demonstrated commendable efficacy, boasting a retention rate surpassing 97% for the entirety of polyphenolic species. Moreover, the employment of the crossflow filtration emerged as a pivotal strategy, profoundly mitigating membrane fouling and culminating in enhanced operational efficiency [70]. Similarly, the concentration of polyphenols derived from elderberry (*Sambucus nigra* L.) juice underwent meticulous examination, encompassing the probing of three distinct commercial NF membranes, each distinguished by varying MWCO thresholds (400 and 1000 Da) and constituting diverse polymeric materials (composite fluoro-polymer and polyethersulphone). All selected membranes showcased commendable rejections concerning a suite of compounds, specifically anthocyanins (cyanidin 3-O-sambubioside and cyanidin 3-O-glucoside), rutin, and astragalin, with rejections exceeding a remarkable 75%. In contrast, relatively diminished rejections were observed concerning catechin and protocatechuic acid, ranging from 25% to 42%. This systematic investigation engenders pivotal insights into the selective retentive capacities of NF membranes, rendering valuable knowledge for optimizing the concentration of polyphenolic species in elderberry juice, and contributing to the burgeoning realm of membrane-based processing strategies for enriching bioactive compounds in functional food products.

The NP030 membrane, characterized by a polyethersulfone composition and an MWCO of 400 Da, exhibited superlative polyphenol rejection capabilities relative to the other two NF membranes under scrutiny, as documented by Tundis et al. [71]. In a separate investigation aimed at polyphenol separation, which encompassed a diverse array of compounds such as anthocyanins, ellagic acid, phytoestrogenic flavonoids, and tannins, sourced from pomegranate juice, a variety of UF and NF membranes sporting nominal MWCOs ranging from 1000 to 4000 Da were rigorously examined. Notably, the Desal GK membrane, with an MWCO of 2000 Da, registered higher productivity, a lower fouling index, and outstanding cleaning efficiency when compared with other membranes. The

retentate stream produced yields of polyphenols and anthocyanins as high as 84.8% and 90.7%, respectively, as reported by Conidi et al. [72]. In a recent foray into the realm of NF processes to concentrate polyphenols from pomegranate peel, a study by Papiannou et al. [73] experimented with an optimal membrane performance under a pressure of 10 bar and a pH of 6, demonstrating a robust polyphenol retention rate as high as 98%.

Investigating pequi (*Caryocar brasiliense Camb.*), a typical Brazilian fruit, Machado et al. [74] revealed that an aqueous pequi extract exhibited an impressive 97% retention of polyphenols, while a 95% ethanol extract exhibited a relatively modest retention of 15%, with the disparity ascribed to the hydrophilic character of the NF90 membrane. Drawing upon the potential of NF for concentrating bioactive compounds from watermelon (*Citrullus lanatus*) juice, Arriola et al. [75] recorded a commendable flavonoid recovery rate of 96%. Similarly, phenolic compounds derived from the seeds of jamun (*Syzygium cumini* L.) fruit, indigenous to India, were effectively purified and concentrated through an integrated approach employing UF and NF membranes, as exposed by Balyan and Sarkar [76].

In an interesting study, Uyttebroek et al. [77] used a commercial NF membrane, NFX, to concentrate polyphenols from apple pomace, yielding an exceptional average retention rate of 98–99% for most polyphenols, e.g., catechin (83%) and epicatechin (93%), attributable to their relatively low molecular weights (290 Da) and the MWCO range of 150–300 Da of the NFX membrane.

Venturing into the domain of anthocyanin-rich jussara (*Euterpe edulis*) fruit extracts, indigenous to Brazil, a comprehensive NF investigation using six commercial flat-sheet membranes with nominal MWCOs spanning from 150 to 1000 (NF270, NF90, NP010, NP030, Desal 5-DK, and Desal 5-DL) unveiled noteworthy insights. NF270, NF90, Desal 5-DK, and Desal 5-DL membranes exhibited equivalent prowess in retaining cyanidin 3-O-rutinoside, and cyanidin 3-O-glucoside, while NP010 and NP030 membranes showcased elevated selectivity exclusively for cyanidin 3-O-rutinoside. Among them, the Desal 5-DK membrane showcased the pinnacle of anthocyanin retention capacity, impressively reaching 98%, as documented by Vieira et al. [78].

Artichoke (*Cynara scolymus* L.) represents a bountiful reservoir of polyphenolic compounds, including apigenin and luteolin, as well as their 7-O-glucosides. The artichoke processing industry generates substantial by-products, such as leaves, external bracts, stems, and blanching waters, constituting nearly 80–85% of the total plant biomass. These by-products are conventionally relegated to animal feed or waste, yet their potential as a reservoir of polyphenols has been brought to light [79,80]. To this end, Conidi et al. [80] undertook a profound exploration of a cutting-edge integrated membrane process, involving UF and NF techniques, designed to separate polyphenols from the artichoke processing industry. Employing the hollow fiber membrane (DCQ III-006C) in the UF stage effectively removed suspended solids, yielding a UF feed with notably elevated apigenin 7-O-glucoside levels (100 mg/L) in comparison to the initial juice (61 mg/L). In subsequent stages, two distinct NF membranes, namely NP030 and Desal DL, were employed to discriminate between polyphenols and sugars. The permeate from the Desal DL membrane exhibited a complete absence of apigenin 7-O-glucoside, while the NP030 membrane displayed a commendably lower rejection rate (82%) for this compound. This process culminated in the production of a polyphenol-enriched retentate, thus opening avenues for its utilization in the realms of food, nutraceutical, or cosmeceutical factories [81]. Furthermore, Conidi and Cassano [82], as part of investigating extract fractionation with membranes, employed a sequential arrangement of two NF membranes (NP030 and Desal DK) to effectually partition polyphenols from aqueous artichoke extracts. Operating under optimized conditions (4 bar, 25 °C), the NP030 membrane exhibited superlative rejections of apigenin, exceeding 85%. Subsequently, the permeate from the NP030 membrane underwent processing with the Desal DK membrane, resulting in the generation of a polyphenol and sugar-free stream. This stream holds promise for potential reuse in irrigation or recycling within the artichoke processing industry [82]. The same research group, as elucidated in a subsequent study [83], adopted an innovative approach that combined membrane processes (UF serving as pre-

treatment, succeeded by NF) with the application of polymeric resins to selectively purify polyphenols from artichoke wastewaters. In the aftermath of UF, the permeate exhibited a marginal 6% decline in apigenin 7-O-glucoside content compared to the feed solution, with NF eliminating polyphenols. Among the diverse macroporous resins scrutinized, S7968 displayed an astounding 100% adsorption ratio for apigenin 7-O-glucoside, closely trailed by S6328 (99.9%) and S2328 (85.7%). Notably, the relatively lower adsorption ratio observed for the S2328 resin was attributed to the discernibly feeble affinity of the analyzed compounds for cation exchangers [83]. In a complementary venture, Cassano et al. [83] undertook an in-depth exploration of flavonoid recovery from artichoke brines, employing five distinct commercial spiral-wound NF membranes (NP010, NP030, NF200, Desal DL, and Desal DK) crafted from various polymeric materials, including polyethersulphone and polyamide, and featuring distinct molecular weight cutoffs (MWCOs) ranging from 200 to 1000 Da. The results showcased the superiority of NF membranes with an MWCO of 200 Da, successfully recovering over 92% of the flavonoids [84]. In another pioneering work, Rabelo et al. [85] examined a sequential process combining ultrasound extraction and advanced membrane technology to recover polyphenols from artichoke solid wastes. A systematic evaluation of different solvent compositions (ranging from 0 to 75% ethanol), ultrasound power levels (ranging from 0 to 720 W), and NF membranes (NF270, DK, and DL) led to the identification of optimal conditions, specifically 50% ethanol, 240 W ultrasound power, and NF employing the DK membrane, which yielded the highest polyphenol recovery [85].

In addition to fruits and vegetables, the utilization of NF processes for polyphenol extraction extends to several other plant sources. Among these is mate (*Ilex paraguariensis* A. St. Hil), a significant plant hailing from the subtropical regions of South America, known for its wealth of biologically active compounds, including flavonoids [86]. In in-depth investigations by Prudencio et al. [87], the core of the research was focused on concentrating major polyphenols extracted from mate bark using NF technology. Impressively, the study concluded that 99% of the polyphenols were successfully retained in the concentrates, indicating the efficacy of nanofiltration in this context. Similarly, the extraction of anthocyanin extract from roselle (*Hibiscus sabdariffa* L.) was subject to scrutiny. Cisse et al. [88] tested ten NF flat-sheet membranes and eight tight UF membranes featuring varying nominal molecular weight cutoffs (0.2–150 kDa). The results demonstrated that all the examined NF membranes exhibited noteworthy retention rates of total anthocyanins, ranging from 93% to 100%.

Moreover, Tylkowski et al. [89] ventured into the concentration of flavonoids from *Sideritis* ssp. L, an endemic plant found in the Balkan Peninsula, using three distinct NF membranes (StarmemTM 240, DuramemTM 300, DuramemTM 500). The study showcased the complete rejection of polyphenols, including flavonoids, by the DuramemTM 300 membrane. Notably, the separation of flavonoids from low-molecular-weight compounds was attainable when the molecular weight cutoff exceeded 400 Da. Eucalyptus bark also emerged as a viable candidate for polyphenol concentration, and Pinto et al. [90] investigated two UF membranes (JW and PLEAIDE) and one NF membrane (SolSep 90801). All the examined membranes displayed selective retention of polyphenols, with the JW membrane exhibiting the highest enrichment of formaldehyde-condensable tannins (17%) and proanthocyanidins (28%). The focus on flavonoid concentration using NF membranes extended to cocoa and propolis as well. Sarmento et al. [90] undertook the concentration of polyphenols from cocoa seeds using commercial NF polymeric membranes (DL, HL, NF, and NF-90). The study unveiled polyphenol rejections ranging from 80% to 95% for the tested membranes. For propolis, a mixture produced by bees, the NF process proved effective in concentrating flavonoids and other phenolic compounds from both aqueous and ethanolic extracts. Mello et al. [91] reported that the NF90 membrane retained approximately 99% of flavonoids in the aqueous solution and 90% in the ethanolic solution. Further investigations by Tylkowski et al. [92] also evaluated the sequential concentration of propolis extract using two membranes (Starmem TM 122 and Duramem TM 200), achieving

remarkable rejections of over 95% with the Duramem TM 200 membrane. Additionally, the same researchers, in a study by Tsibranska et al. [93], employed membranes with varying molecular weight cutoffs (300–900 Da) to fractionate flavonoids from propolis extract. The rejections for total polyphenols and flavonoids ranged from 30% (900 Da membrane) to an impressive 94% (300 Da membrane), demonstrating the versatility and potential of NF in the concentration and fractionation of polyphenols from various plant sources [94].

2.2. Non-Flavonoids

As for flavonoids, their extraction has also received attention from distinct natural products and by-products, as reported in Table 2. For instance, artichoke (*C. scolymus* L.) stands as another compelling source of polyphenols, encompassing not only flavonoids but also phenolic acids, most notably chlorogenic acid and cynarin. Conid et al. [81] delved into the separation of polyphenols from artichoke wastewaters using two distinct NF membranes (NP030 and Desal DL). The study unveiled that the Desal DL membrane permeate lacked phenolic acids, whereas the NP030 membrane exhibited a lower rejection towards chlorogenic acid (95%) and cynarin (90%) [81]. A parallel inquiry by the same research group applied the NP030 and Desal DK membranes sequentially for the separation of phenolics from aqueous artichoke extracts, resulting in high rejections exceeding 85% for chlorogenic acid and cynarin [83]. Similarly, the same researchers employed a combination of membrane processes and polymeric resins to achieve phenolic purification from artichoke wastewaters, with the S7968 resin demonstrating the highest adsorption ratio for chlorogenic acid (81.35%) [84]. They expanded this exploration to the recovery of phenolics from artichoke brines, demonstrating that NF membranes of 200 Da recovered over 92% of total hydroxycinnamic acids, chlorogenic acid, and cynarin [84]. Correspondingly, another study harnessed the efficacy of nanofiltration in purifying tyrosol in olive mill wastewaters, revealing the separation of almost all phenolics after optimized NF conditions [95].

Table 2. Some relevant nanofiltration applications for the recovery of non-flavonoids.

Material	Membrane Specifications			Selected Membrane	Polyphenol Rejection	Reference
	Membrane	**Polymer**	**MWCO (Da)**			
Olive products	n/a	n/a	n/a	Integrated membrane system	≈100%	[96]
	Nadir N30F	Polyethersulphone	578			
	DK	Polyamide/Polysulfone	150	MPF44	OLP: 96%	[97]
	DL	Polyamide/Polysulfone	300			
	G10	Polyethylene glycine	2500			
	G5	Polyethylene glycine	1000			
	MPF34	Silicone/Polysulfone	200			
	MPF36	Silicone/Polysulfone	1000			
	MPF44	Polyvinyl alcohol	250			
	NTR7250	Sulfonated polyether sulfone	300–400			
	NTR7410		17,500			
	NTR7430	Sulfonated polyether sulfone	2000			
	NTR7450		700–800			
	Isoflux	Sulfonated polyether sulfone	n/a	Integrated membrane system	85%	[98]
	n/a	TiO_2	n/a			
	DL1812	TiO_2	n/a			
	NF90	Polyamide	150–300			
	LiquiCells ExtraFlow	Polyamide	200			
	Shirasu porous glass	Polypropylene	n/a			
		Al_2O_3 SiO_2 glass	n/a			

Table 2. *Cont.*

Material	Membrane Specifications			Selected Membrane	Polyphenol Rejection	Reference
	Membrane	**Polymer**	**MWCO (Da)**			
	n/a	Polypropylene	n/a	Integrated membrane system	78.3%	[99]
	UF(ARS)	Polysulfonamide	20,000			
	NF-70	Polyamide	200			
	TOPER	n/a	n/a	Integrated membrane system	HT: 100% SA: 100% OLP: 100%	[100]
	ZeeWeed 1500	n/a	5000			
	HL2540TF	n/a	300			
	MD 020 TP 2N	Polypropylene	n/a			
	GK	Polyamide	3500	Integrated membrane system	HCA: 100%	[101]
	GH	Polyamide	2500			
	GE	Polyamide	1000			
	NFA-12A	Polyamide	500			
	DK	Polyamide	150–300			
	NF270	Polyamide	300 Da	NF270	≈100%	[95]

n/a: Not Applicable.

Beyond olive and artichoke by-products, the NF process has been extensively explored for concentrating phenolic compounds from diverse sources. Cai et al. [102] navigated the recovery of phenolic compounds from fruit juices using two NF membranes (VNF1 and VNF2) composed of distinct polymeric materials (polyamide and polypiperazine amide) and featuring different molecular weight cutoffs (240 and 150 Da). This undertaking resulted in rejections ranging from 18.91% to 96.80% and 39.69% to 98.23% for gallic, protocatechuic, caffeic, ferulic, and chlorogenic acids with VNF1 and VNF2, respectively [102]. Venturing further, the concentration of phenolic compounds from the pequi fruit native to Brazil was undertaken, employing sequential MF, UF, and NF0 processes, resulting in the recovery of over 80% of ellagic acid and 95% of p-coumaric acid [103].

Additionally, Murakami et al. [85] experimented on the concentration of phenolic compounds in mate extract through nanofiltration processes, with impressive recoveries ranging from 95% to 100% for 4,5-dicaffeoylquinic, gallic, 3,4-dihydroxybenzoic, and chlorogenic acids. In a distinct investigation, a different scholarly endeavor harnessed the potential of three distinct nanofiltration membranes, namely DuramemTM 200, DuramemTM 300, and DuramemTM 500, to facilitate the concentration of phenolic compounds derived from rosemary (*Rosmarinus officinalis* L.). Among this triad, the DuramemTM 200 membrane emerged as the star performer, boasting an exceptional performance characterized by robust rejections ranging from 93.9% to 96.1% for caffeic acid and a striking 99.5% to 99.7% for rosmarinic acid [104].

In a parallel scientific endeavor, Achour et al. [105] worked on the concentration of phenolic compounds extracted from *Thymus capitatus* through the strategic implementation of membrane processes. The consequential findings illustrated a compelling distinction, revealing that the synthetic NF membrane outperformed both its commercial NF and synthetic UF counterparts in the concentration of phenolic compounds within the retentate fraction [105].

3. Nanofiltration Membranes for the Extraction of Carotenoids

Carotenoids, distinguished as tetraterpenoids comprising 40 carbon atoms, manifest as the red, yellow, or orange pigments intrinsic to a plethora of fruits and vegetables. This classification bifurcates into carotenes and xanthophylls. Carotenes and xanthophylls, as polyenic hydrocarbons, exhibit variable degrees of unsaturation, with xanthophylls arising from carotenes through hydroxylation or epoxidation reactions. Notable examples encompass β-carotene, lycopene as carotenes, and lutein, zeaxanthin within the xanthophyll cohort [106,107].

The pronounced nutritional significance of β-carotene is well-acknowledged, given its capacity to serve as a pivotal precursor for vitamin A synthesis within the human body [108]. This dynamic feature positions it as a prospective avenue for addressing widespread vitamin A deficiency, which a common concern in society [109]. Furthermore, an assemblage of research has substantiated the potential of carotenes in mitigating certain cancers, including gastric, pulmonary, oral, and pharyngeal malignancies [110]. Beyond this, carotenoids exhibit an immune-boosting potential, rendering protection against influenza, bronchitis, infections, and toxins. In addition, these compounds hold substantial dyeing capabilities, facilitating the impartation of desired color traits to food products even at minute concentrations, mere parts per million [111]. The versatility and allure of carotenoids have markedly escalated within the cosmetics, pharmaceutical, and food sectors, underscoring the pivotal role of their extraction from food sources or by-products.

Several foods emerge as notable reservoirs of carotenoids, encompassing squash, carrots, pineapples, sweet potatoes, lettuce, mustard, and kale, among others. In tandem, distinctive local sources endowed with copious β-carotene content include Brazilian wine palm (*Mauritia flexuosa*), palm fruit (*Elaeis guineensis*), tucuma (*Astrocaryum aculeatum*), macaúba (*Acrocomia aculeata*), pupunha (*Bactris gasipaes*), and pequi (*Caryocar brasiliense*) [105]. Beyond the plant kingdom, carotenoids also grace the realms of bacteria, fungi, and algae [107].

Despite their functional attributes, carotenoids evince susceptibility to environmental influences, spanning light, temperature, acidity, and oxidative reactions attributed to their unsaturated bonds. Their lipophilic nature renders them water-insoluble yet soluble in solvents such as acetone, alcohol, and chloroform [106,112]. Regrettably, conventional physical and chemical methodologies for palm oil processing typically result in the extraction and loss of nearly all carotenes. Remediation strategies recommend an additional membrane filtration stage prior to the chemical or physical processing of crude palm oil, enabling carotenoid retrieval. Among different separation techniques, NF emerges as an efficacious avenue for extracting carotenoids from crude palm oil, leveraging its energy efficiency, ambient operating conditions, and the preservation of thermosensitive constituents. In the landscape of organic solvent nanofiltration (OSN), diverse applications encompassing catalytic recovery, solvent exchange, chiral isolation, natural extract concentration, and peptide synthesis have burgeoned. While studies investigating membrane technologies for carotenoid retrieval from palm oil are limited, existing research revolves around a sequence wherein palm oil is first trans-esterified into methyl esters, subsequently followed by NF-driven carotene extraction from these esters [7,113,114].

NF holds considerable promise for the concentration of phenolic fractions and carotenoids within intricate solutions. Its capacity for discerning compounds based on nuanced differentials in molecular dimensions and electrostatic attributes renders it advantageous for the isolation of these bioactive constituents from heterogeneous matrices wherein they may cohabit with diverse molecular entities [115]. The operational parameters of NF, characterized by low pressures and ambient temperatures, are positive to the preservation of the structural integrity of delicate phenolic fractions and carotenoids [116]. This feature becomes particularly salient when considering the susceptibility of these compounds to structural degradation under more stringent processing environments. Additionally, the low energy consumption of NF makes it a sustainable and eco-friendly concentration process.

However, NF still poses inherent challenges that merit scrupulous consideration. Foremost among these is the specter of membrane fouling, a pernicious phenomenon wherein contaminants accrue on the membrane substrate, precipitating a gradual attenuation in filtration efficacy. For phenolic fractions and carotenoids, compounds often characterized by intricate molecular architectures, membrane fouling emerges as a formidable impediment, necessitating a regimen of periodic maintenance and rigorous cleansing protocols [117].

Given the dimensions of the solutes and electrostatic attributes of these compounds, fine-tuning process parameters and membrane characteristics is crucial to achieving the

desired separation efficiency. This accentuates the complexity inherent in the pursuit of precise separation within the nanoscale domain [117].

The economic calculus governing NF processes also introduces a dimension of complexity, particularly in the realm of cost considerations. The initial capital outlay and the exigencies of maintaining high-selectivity membranes engender economic challenges, particularly in scenarios marked by resource constraints [116]. Exposure to deleterious chemical agents and elevated pressures may engender membrane degradation, thereby diminishing the longevity of said membranes and concomitantly escalating the financial outlay associated with their subsequent replacement [117]. In addition to this, it is important to consider that NF necessitates a substantial energy expenditure attributable to the imperative deployment of high-pressure pumps and the utilization of substantial water volumes for cleansing and maintenance. Hence, the cost–benefit relationship is essential to ascertain the economic viability of NF in the context of phenolic fraction and carotenoid concentration.

The financial outlay associated with the procurement of NF membranes and accompanying equipment may reach elevated levels, thereby imposing a potential constraint on the extensive integration of this technology within specific industries. Furthermore, the scalability of NF processes is circumscribed by specific factors, including membrane fouling dynamics and the requisite deployment of intricate equipment. Scaling up the process necessitates a strategic equipoise, entailing additional investments to efficaciously address the challenges germane to membrane fouling and perpetuate overarching process efficiency [116].

In summation, while NF distinguishes itself with its well-designed molecular selectivity in concentrating phenolic fractions and carotenoids, some drawbacks, including membrane fouling, selectivity drops, economic considerations, and scalability concerns, are imperative for the optimization of its practical deployment across diverse scientific and industrial scales.

3.1. Carotenoids

Lycopene has garnered escalating research interest due to its favorable health impacts and robust lipophilic antioxidant attributes within tomatoes [118]. Among its documented benefits are cardiovascular safeguarding [119], myocardial infarction risk mitigation [120], blood pressure reduction [121], prevention of LDL cholesterol oxidation [122], lowered susceptibility to prostate cancer [123], lung cancer [124], ovarian cancer [125], and breast cancer [126], and potential amelioration of neurodegenerative diseases like Alzheimer's and Parkinson's [127]. These health effects are primarily attributed to lycopene's antioxidant properties [128].

NF presents several advantages, notably the retention of smaller particles compared to MF and UF. Arriola et al. [75] investigated NF for concentrating bioactive compounds in watermelon juice, highlighting lycopene's pronounced rejection coefficient (0.99). This supports NF as an effective strategy for concentrating key bioactives in watermelon juice [75].

Luo [128] explored lycopene concentration via solvent-resistant NF membranes using Starmem 122, Starmem 240, and Duramem 300. Membrane efficacy was determined through rejection and permeation rates, supplemented by SEM and FTIR analyses pre- and post-NF. Notably, Starmem 240 demonstrated favorable lycopene concentration, achieving a twofold to threefold increase over the feed concentration. Although corrosion induced permeability decline, ultrasonic dipping in petroleum countered this challenge. Recycling permeate solvent brings economic and environmental gains, further supported by membrane stability post-permeation, as observed through SEM and FTIR [129]

Arana et al. [129] conducted a study on lycopene concentration and purification from tomatoes and tomato juice via membrane technology and solvent extraction. Five polymeric membranes were assessed with hexane lycopene extracts, indicating a potential five-step NF process to recover 90.2% of lycopene, resulting in a final retentate stream of over 157 mg/L lycopene and a recyclable permeate stream of 3.6 mg/L lycopene. Economic

analysis affirmed the viability and efficacy of industrial lycopene recovery using membrane technology [130].

β-Carotene (BC), a lipophilic carotenoid prevalent in vegetables and fruits, holds provitamin A status due to its in vivo conversion to vitamin A. BC's anticipated pharmacological actions encompass skin and mucous membrane maintenance and improved visual acuity [131]. Additionally, BC's robust antioxidant activity aids in quenching excess in vivo reactive oxygen species, potentially preventing degenerative diseases such as cardiovascular diseases, diabetes, and specific cancers [132]. Consequently, BC finds application in pharmaceuticals, dietary supplements, and cosmetics, warranting attention to its recovery from foods and by-products.

The prospect of integrating supercritical CO_2 extraction (SC-CO_2) with NF stages for extracting and purifying low-molecular-weight compounds (up to 1500 g mol^{-1}) has previously been advanced. The Commissariat à l'Énergie Atomique (CEA) has developed two NF tubular membranes fortified against supercritical conditions, primarily tailored for liquid filtration to segregate low-molecular-weight compounds (500 to 1000 g mol^{-1}). The first membrane presents as a multilayer composite nanofilter, comprising a macroporous aluminum substrate, a mesoporous titanium oxide substrate, and an organic NafionA upper layer. The inorganic material provides mechanical stability to the composite NF membrane, while the organic polymer provides selectivity. The other membrane follows a strictly inorganic design, consisting of a macroporous alumina substrate coated with a titanium oxide layer synthesized via the sol–gel route. This titanium oxide coating enhances thermal and chemical resistance. Given its favorable physico-chemical properties and low critical point (7.38 MPa and 304.2 K), carbon dioxide emerges as the chosen supercritical fluid for extraction [62].

In the pursuit of refining *β*-carotene from either carrot oils or carrot seeds, Sarrade et al. [133] embarked on an investigation. This pigment, extensively utilized in the agro-food and cosmetic sectors, is notably sensitive to both temperature and oxidation, thereby engendering isolation challenges. The conjoint methodology yielded promising outcomes in terms of segregation and refinement. In the context of *β*-carotene purification from carrot oil, the employment of T membranes resulted in a 2.4-fold enhancement in pigment concentration within the permeate. In the treatment of carrot seeds, predominant purification was achieved through the CO_2 stage, yet the employment of TN membranes yielded a 30% augmentation in the NF stage's efficacy [133].

Palm oil emerges as a prolific repository of *α*- and *β*-carotene (400–3500 mg/kg), constituting more than 80% of the collective carotenoid content in palm oil [134]. Following Darnoko and Cheryan [113], palm oil harbors substantial carotenoids and tocopherols that can be extricated through their conversion to methyl esters, in conjunction with the utilization of membrane technology to sequester carotenoids from methyl esters. Multiple solvent-stable NF membranes were evaluated for this purpose. The permeation rate, tested with a model solution of crude palm oil methyl esters, ranged from 0.5 to 10 L/m^2 h, with *β*-carotene retention spanning from 60% to 80%, achieved under a transmembrane pressure of 2.76 MPa at 40 °C. A multi-stage membrane process was proposed to generate concentrated palm carotenoid methyl esters continuously. With a feed rate of 10 tons/h of palm oil methyl esters containing 0.5 g/L of *β*-carotene, the process yielded 3611 L/h of a carotenoid concentrate, bearing 1.19 g/L of carotene, alongside 7500 L/h of bleached methyl esters, containing less than 0.1 g/L of *β*-carotene. Notably, Chiu et al. [7] further developed the concentration of carotenoids from crude palm oil via NF, managing to retain 75% of *β*-carotene. However, it is imperative to acknowledge that this process decreases the nutritional value of the edible oil for subsequent consumption.

Pequi (*Caryocar brasilense Camb.*), which is a traditional Brazilian fruit, boasts a wealth of polyphenols and carotenoids. The analysis by Machado et al. [114] encompassed both aqueous and alcoholic pequi extraction, evaluated at a bench scale, discerning the influence of temporal and thermal variations on the retrieval of polyphenols and carotenoids. Optimal extraction conditions, 25 °C over 1 h for the aqueous extract and 40 °C spanning 24 h for

the alcoholic extract, were determined. A re-extraction of residues ensued, enhancing the compound retrieval from the fruit. The resultant extract, a fusion of the initial and subsequent extracts, underwent a concentration phase through NF, utilizing a stirred cell at 25 °C and 800 kPa pressure. In the realm of the alcoholic extract, the rejection of bioactive compounds was confined to approximately 10% for carotenoids and 15% for polyphenols. NF showed a robust efficiency in concentrating polyphenols and carotenoids within the aqueous extract, reflected by a retention coefficient of around 100% and 97%, respectively [74].

In search for the preservation of phenolic fractions and carotenoids, lyophilization can be used for this purpose, as inn the study published by Garcia-Martinez et al. [135], which evaluated the effect of lyophilization on the bioactive compounds of orange juice co-products. The study found that lyophilization can preserve the bioactive compounds of orange juice co-products, including carotenoids and phenolic compounds. Overall, lyophilization can be a useful technique for the preservation of phenolic fractions and carotenoids, However, lyophilization can also lead to the degradation of these bioactive compounds due to the high temperatures and pressures involved in the process. Therefore, it is important to optimize the lyophilization process to minimize the loss of these compounds.

Mussagy et al. [136] integrated a methodology for the recovery, refinement, and polishing of torularhodin, β-carotene, and fatty acids extracted from biomass samples of *R. glutinis* CCT-2186. Noteworthy is the discernible enhancement in recovery yields, particularly in dry biomass, consequent to a single freeze/thaw cycle pre-treatment. The integrated process, incorporating solid–liquid extraction (SLE) for cell disruption and subsequent liquid–liquid extraction (LLE) for separation and polishing, facilitates efficient and selective isolation of carotenoids from fatty acids. Moreover, the sustainable reutilization of biosolvents for up to three cycles underscores the cost-effectiveness and environmental viability of this approach.

Encapsulation emerges as an additional strategy poised to counteract the innate vulnerability of carotenoids to oxidation, thereby presenting a formidable challenge to their stability and, consequently, impeding their effective integration within the food industry. This methodology seeks to mitigate the exposure of carotenoids to deleterious oxidative agents, including elevated temperatures, atmospheric oxygen, acidic milieu, and catalytic metal ions. By encapsulating carotenoids within protective matrices, this approach effectively shields them from the detrimental effects of oxidation, thus preserving their functional attributes and enhancing their viability for diverse food applications. Among these techniques, microencapsulation, nanoencapsulation, and supercritical encapsulation have emerged as new solutions to these intricate issues. By entrapping carotenoids within protective matrices at the micro- or nano-scale, these methodologies impart heightened stability, enhanced solubility, precisely controlled release kinetics, and augmented bioavailability. Consequently, the transformative potential of encapsulation technologies propels an unprecedented expansion in the application of carotenoids for different food formulations [137]. Bazzarelli et al. [98] introduced an innovative process design for encapsulating polyphenols extracted from olive mill wastewaters, combining conventional NF with emerging membrane techniques such as osmotic distillation and membrane emulsification. Following the removal of suspended solids via an acidification/MF procedure, the MF permeate underwent NF treatment to partition water into the permeate while yielding a concentrated polyphenolic solution in the retentate. Remarkably, the MF process exhibited minimal rejection rates towards phenolic compounds, approximately 6.8%, ensuring their effective recovery within the permeate stream.

Nanoencapsulation technology presents the capacity to yield more stable products boasting superior absorption and bioavailability. Various carriers, including nanoemulsions, nanoliposomes, solid lipid nanoparticles, and nanostructured lipid carriers, facilitate the encapsulation of carotenoids [138], characterized by minimal phase segregation, preserved bioactive properties, improved absorption and bioavailability, limited interaction between bioactive compounds and other food components, and reduced impact on sen-

sory attributes. Nanoencapsulation techniques encompass both top-down and bottom-up methods for nanoparticle production. Bottom-up techniques entail the self-organization and self-assembly of molecules through processes such as nanoprecipitation, coacervation, and inclusion complexation. Conversely, top-down techniques necessitate specialized equipment to diminish particle size and yield nanostructures. Techniques in this category comprise extrusion, homogenization, electrospinning/spraying, and emulsification–solvent evaporation processes [139,140].

Regarded as the predominant method for encapsulating carotenoids, microencapsulation is distinguished by its straightforward techniques and capacity to yield high-quality products, despite its tendency for diminished stability over time. This method enables the swift production of stable powders within a size range of 1 to 1000 μm, achieved at low cost and temperature. Microencapsulation facilitates the encapsulation of thermolabile compounds and stabilization of the encapsulated substance through the utilization of common carriers. These carriers encompass a variety of polysaccharides (e.g., maltodextrin, starch, chitosan, inulin, sodium alginate, carrageenan, pectin, carboxymethyl cellulose, and citrus fibers), gums (e.g., Arabic gum, Mesquite gum, Guar gum, and locust bean gum), and proteins (e.g., gluten, casein, gelatin, whey protein, soy protein, albumin, milk powder, and oligopeptides) [137,141,142].

Alternatively, green technologies, such as supercritical encapsulation, emerge as promising alternatives for micro- and nanoencapsulation, particularly for thermolabile compounds like carotenoids, without compromising sensory attributes, making them suitable for application in the food industry. Supercritical carbon dioxide (SC-CO_2), renowned for its low critical point (Tc = 31.10 °C, Pc = 7.38 MPa), safety, low viscosity and reactivity, easy elimination, ability to inactivate microbes, relatively low cost, and better solubility of some lipophilic compounds, stands out as the most popular supercritical solvent utilized in the food and pharmaceutical industry [142,143].

3.2. Xanthopylls

Lutein along with its stereoisomer zeaxanthin are members of the fat-soluble xanthophylls, a subset of the carotenoid family. Alongside their isomeric counterpart meso-zeaxanthin, these xanthophylls constitute the primary constituents of macular pigment. Following ingestion, this compound becomes concentrated within the macula region of the retina, playing a pivotal role in fine-feature vision—an attribute distinguishing them from other natural carotenoids [144]. Notably, a decline in the macula and lens accumulated lutein and zeaxanthin has been associated with the onset of cataracts and age-related macular degeneration, both of which contribute to blindness [145].

Xanthophylls, also known as oxo-carotenoids, feature at least one oxygen atom within their structure. Lutein (β, ε-carotene-3,3′-diol) and zeaxanthin (β,β-carotene-3,3′-diol) stand as dihydroxy derivatives of α- and β-carotene, respectively, with hydroxyl groups adorning the 3 and 3′ positions of their ionone rings (Figure 4). This hydroxyl inclusion imparts a greater polarity to these compounds compared to standard carotenoids. The antioxidant efficacy of carotenoids is modulated by their conjugated double bond arrangement, which concurrently dictates their light absorption properties. While the double bonds within the rings of lutein and zeaxanthin exhibit partial conjugation, the nine C-C double bonds in their polyene backbone remain fully conjugated. Their respective maximum absorption wavelengths approximate 445 nm and 450 nm, with molar extinction coefficients (ε_{mol}) spanning from 140,000 to 145,000 cm^{-1} mol^{-1} [146]. This polarity and conjugated double bond structure endows them with notable free radical scavenging capabilities [147].

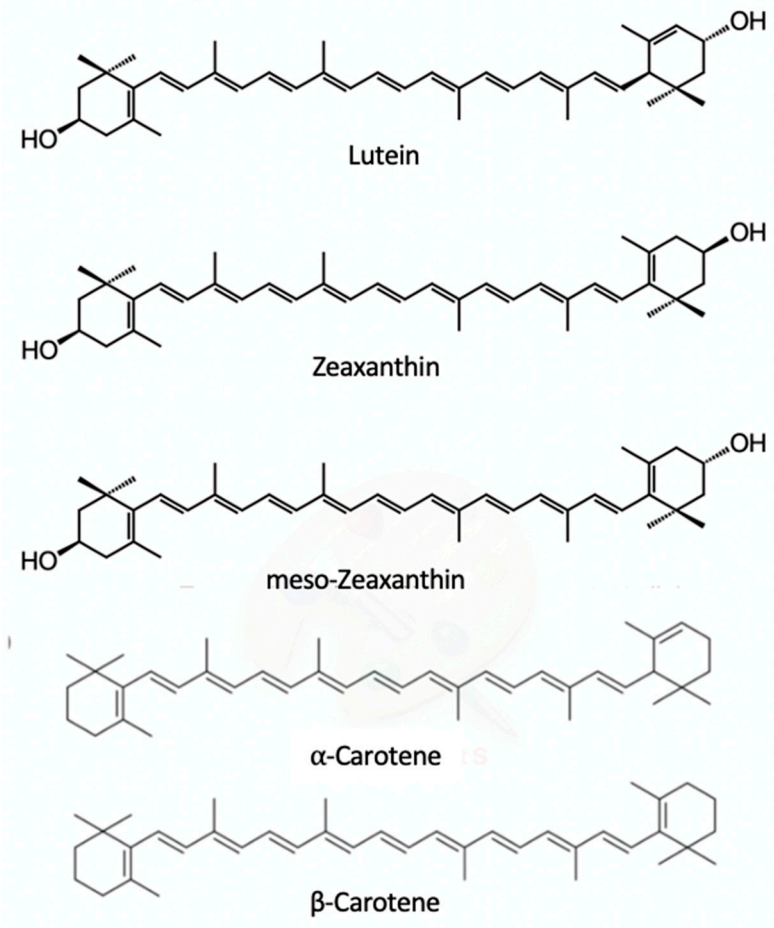

Figure 4. Chemical structures of main xanthopylls and carotenoids.

Various studies have indicated their potential in cancer prevention, including lung, colorectal, ovarian, and breast cancer, as well as coronary heart disease. Moreover, they have the capacity to absorb and attenuate blue light, a significant factor in retinal damage from excessive exposure [148,149]. It is unsurprising that these xanthophylls assume a pivotal role in sustaining optimal vision, concentrating within the macula and lens, both vulnerable to oxidative harm due to intense light exposure. However, despite their structural resemblance to α- and β-carotene, they lack provitamin A activity [148].

Lutein and zeaxanthin are abundant in numerous fruits and vegetables. While lutein is predominantly extracted from marigold flowers (*Tagetes genus*) in the global market, reliance on periodic flower harvesting has prompted exploration into alternative sources, including microorganisms like microalgae. Examples of microbial sources encompass *Flavobacterium* sp., *Synechocystis* sp., *Spirulina*, *Chlorella fusca*, and *Chlorococcum citroforme* [150,151]. Notably, microalgae are gaining attraction due to their elevated lutein and zeaxanthin content, coupled with a growth rate 5–10 times faster than higher plants [152].

According to Agricultural Research Service of the US Department of Agriculture [153], lutein and zeaxanthin are present in various foods. Green leafy vegetables, such as spinach or kale, are rich sources of these compounds, although their yellow-orange hue often remains concealed due to the abundance of chlorophyll [154]. Notably, the structures of lutein and zeaxanthin bear close resemblance, differing only in the double bond position within one ionone end ring [155]. Analytical quantification of these compounds within foods presents a challenge. Until recently, their quantification remained unfeasible due to analytical limitations, consequently leading to the presentation of combined lutein and zeaxanthin content in much of the literature [144]. In the broader context, valuable natural compounds tend to be present in minute quantities within their natural sources. Consequently, following the extraction process employing a suitable solvent, the resultant

extract is highly diluted, necessitating subsequent stages of separation, concentration, and purification. Among these steps, purification emerges as the most intricate facet within the production of natural compounds. Consequently, there is an interest in developing separation and purification technologies that are selective, sustainable, and energy-efficient for high-value natural products [156]. Organic solvent NF stands as a novel technology that operates based on the molecular weight or size of solutes, falling within the range of 50 to 2000 g mol^{-1}. This technology encompasses solvent recovery and exchange, facilitated through the application of a pressure gradient within the organic medium [157]. Notably, within the existing literature, only one study exists concerning the NF process applied to xanthophylls. Tsui and Chryan [158] were interested in the purification of xanthophylls, extracted from corn via 85% aqueous ethanol, utilizing organic solvent NF. Their investigation indicated the successful purification and concentration of the ethanolic xanthophyll extract through a series of filtration with UF and NF steps. Additionally, the DK membrane, boasting a molecular weight cutoff of 300 Da and manufactured by GE-Osmonics located in Minnetonka, MN, was found to perform better than the other membranes in terms of flux, rejection, and stability attributes.

4. Navigating Challenges in Bioactive Molecule Recovery

The recovery and purification of bioactive compounds strongly depend on the specific attributes of the target biomolecule and its final application. Diverse parameters, encompassing the compound's intrinsic nature, localization, whether intracellular or extracellular, dimensions, configuration, charge, and solubility, among others, are determinants for the purification. Additionally, factors related to the desired degree of purity for the target compound and the preservation of its bioactivity must be considered [159].

Moreover, the incorporation of agro-industrial residues in bioactive compound recovery profoundly influences and the extraction and purification processes. Characteristics of the waste material, including particle size, solubility, viscosity, and resistance to degradation, may present impediments during extraction, cell disruption, and purification phases, thereby impacting the intricacy and number of subsequent purifications. Furthermore, the concurrent generation of multiple bioactive compounds within a singular medium emerges as a pivotal consideration, thereby complicating purification scheme formulation and exerting influence over associated costs and operational complexities [6].

The primary challenge with the utilization of NF is membrane fouling, where contaminants are accumulated on the membrane surface, impeding filtration efficiency. To address this, innovative strategies, such as membrane modification with fouling-resistant materials or surface alterations, can mitigate fouling effects and extend membrane lifespan [160,161]. Several studies have demonstrated the effectiveness of surface modification in improving fouling resistance. For instance, a study published by Cheng et al. [162] reported that hydrophilic membrane surface modification can mitigate irreversible foulant deposition, thereby improving fouling resistance. Similarly, Taghavian et al. [163] highlighted the role of membrane surface treatment in modifying anti-fouling resistivity, particularly in water treatment applications. Furthermore, a study by Choi et al. [164] evaluated the feasibility of modified membranes and examined the improvement in fouling resistance after surface modification. This study documents the effective application of air-stimulated surface polymerization of dopamine hydrochloride incorporated with zinc oxide nanoparticles (ZnO NPs) to mitigate the inherent hydrophobicity and limited anti-(bio)fouling resistance of polytetrafluoroethylene (PTFE) hollow fiber membranes (HFMs). Additionally, Ahmad et al. [165] provided an overview of various modification strategies for developing antifouling NF membranes, emphasizing the use of polydopamine (PDA) and antifouling modifiers (nanoparticle, polymer, and composite polymer/nanoparticle), as well as denoting the role of graphene-based nanoparticles, CQD, zwitterionic polymers, and dendrimers, as promising modifying agents able to impart fouling-resistant properties. In another study, Kim et al. [166] discussed the fouling-resistant surface modification of forward osmosis membranes using MoS_2-Ag nanofillers. These findings collectively underscore the poten-

tial of membrane surface modification in enhancing fouling resistance and extending the lifespan of membranes in various applications, including water treatment and NF.

Implementing real-time monitoring systems to detect fouling events and employing automated cleaning protocols can help maintain membrane performance and prevent irreversible fouling. Regular cleaning intervals and the utilization of environmentally friendly cleaning agents further contribute to mitigating fouling effects without compromising membrane integrity, thereby ensuring operational efficacy [160]. Another significant concern lies in achieving precise separation and purification of target bioactive molecules in a complex matrix due to molecular similarities with other constituents. NF's intrinsic selectivity may prove insufficient in achieving the desired separation, risking contamination or loss of valuable compounds. Process optimization emerges as a pivotal solution. Fine-tuning parameters, such as transmembrane pressure, feed flow rate, and pH, allows for the optimization of NF conditions, enhancing selectivity and efficiency. Furthermore, advances in membrane design and material selection offer tailored solutions to specific separation challenges, improving overall performance.

The scalability of NF for the separation of carotenoids is an area of ongoing research and development. Several studies have indicated the potential for scaling up NF processes for the enrichment and separation of carotenoids. For example, a study on the influence of NF membrane features on the enrichment of jussara extract, which contains carotenoids, mentioned the evaluation of the economic feasibility of scaling up the process [167]. Additionally, research on the separation of polyphenols and carotenoids using NF suggested that this technique has established a high separation efficiency for these compounds, indicating its potential for scalability. Furthermore, a study on the recovery of carotenoids from red palm methyl esters by NF discussed the economics of the process, indicating promising potential for scalability [167,168]. While the specific details of the scalability of NF are still evolving, these studies suggest a positive outlook for the scalability of NF processes for the enrichment and separation of carotenoids.

To some extent, navigating the challenges of membrane fouling, selective separation, and scalability in NF for the recovery and purification of bioactive molecules necessitates a multifaceted approach. Using innovative membrane design, process optimization, and strategic operational approaches ensures the emergence of NF as a powerful tool for efficient and sustainable separation processes in various industrial applications.

5. Conclusions and Future Perspectives

Today, within the context of escalating interest in natural compounds boasting remarkable biological activities, pressure-driven membrane-based technologies have emerged as highly effective means for the separation, isolation, and fractionation of bioactive components from food resources and their by-products. Among these techniques, NF membranes have garnered prominent recognition for their unparalleled ability to recover polyphenols and carotenoids from diverse food product categories. Indeed, membrane processes have garnered considerable attention as promising tools for processing fruit juices [169], representing a compelling alternative to conventional methodologies employed for the concentration of bioactive compounds. The appeal stems from their inherent advantages, including high recovery and/or removal efficacy, operation under mild conditions, absence of phase transitions, and minimal energy consumption. Notably, NF offers particularly advantageous opportunities, frequently linked to the preferential preservation of lower-sized particles relative to MF and UF techniques. Over the course of this extensive review, it can be said the latter technologies are needed to assist NF stages in the pretreatment of raw extracts with complex composition [170].

An advantage of the utilization of NF membranes for the concentration of bioactive compounds from food matrices lies in their innate capacity to separate molecules within the 100–1000 Da range. Preliminarily speaking, the adoption of NF processes is anticipated to become increasingly pervasive due to their several advantages. Even more interestingly, these processes are strongly used within the circular economic concepts due to their green

aspects in the environment [171,172]. It is worth mentioning that NF also presents the ability to be integrated into other emerging selective extraction technologies, such as ultrasound-assisted pressurized liquid extraction, reporting a retention extraction over 89% [173].

Nonetheless, it behooves the scientific community to extend the advantages of NF while facing its constraints and applications to attain a more comprehensive understanding of its potentialities [174]. Finally, mostly commercial NF membranes have been investigated in recovering bioactive compounds from natural sources; however, there is a current interest in fabricating composite NF that may offer superior recovery efficiencies [175]. Mostly, the physicochemical properties of membranes are being improved by different scenarios, such as polymer blending [176], inorganic nanomaterial incorporation [177], and post-treatment modification [178]. By modifying the properties of membranes, enhanced separation performance can be obtained if the polymer or inorganic nanomaterials are suitably merged [179]. Therefore, the next generation of membranes for pressure-driven membrane processes will be a scope of study in the near future.

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