

A Review of Microfiltration Membrane in Separating Folic Acid from Fermented Dark Green Leafy Vegetables (DGLVs), Legumes, Beans, and Cereal for Nutraceutical Application

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Abstracts: DGLVs (spinach, broccoli), legumes (soybean, mung bean, kidney bean), and cereal (corn) are well recognized for human health benefits due to the presence of natural folate (vitamin B9) with essential micronutrients. Folate is among the most unstable vitamins for the normal functions of the human body. The recent development of membrane-based methods for the separation of folic acid (FA) is reviewed from the selected literature and covers the result of significant research that has been performed in the last five years on the use of the microfiltration (MF) method. This is coupled with the fermentation process to support and achieve separation or recovery of FA from their selected plant-based food source. The nixtamalization, fermentation, separation, and purification of DGLVs, legumes, and beans, as well as corn, were conducted using the MF (pore size 0.15 μm) membrane separation method. The results showed that the MF membrane was able to separate FA components in permeate from fermented spinach and broccoli, soybean, mung bean, kidney bean, and corn, namely 7.14 and 3.79, 197.00, 325.37 and 242.93, and 121.10 $\mu\text{g/mL}$, respectively. Meanwhile, FA content in retentate from fermented spinach and broccoli, soybean, mung bean, kidney bean, and corn was 58.90 and 28.10, 362.07, 254.07 and 506.07, and 212.84 $\mu\text{g/mL}$, respectively. The applications of membrane techniques were used as an alternative to some unit operations in the solving of separation issues as well as the development of selected new fermented plant-based food sources. The contemporary pressure-driven membranes, such as MF, and the main applications were related to pre-treatment, selected fermented plant-based food sources, and alternative technology for extending the shelf life of natural folate. The utilization of MF membranes in the nutraceutical field holds significant promise, particularly in the creation of value-added products from minor compounds.

Keywords: Folate, DGLVs, Legume, Corn, Nixtamalization, Fermentation, Microfiltration (MF).

1. INTRODUCTION

1.1. Folate

The generic term for folate and its derivatives is a family of B vitamins, specifically Vitamin B9, having similar biological activity. Folate can have several different forms containing a pteroyl group, which is an essential water-soluble molecule existing as tetrahydrofolate (THF, the active and natural form) and methyltetrahydrofolate (MTHF, primary form found in blood). Meanwhile, folic acid (FA) is an inactive form in the human body and has to be converted by the liver into an active molecule, 5-methyltetrahydrofolate (5-MTHF). FA (CAS Number 59-30-3; pteroyl-glutamic acid, folate, folacin) has a molecular formula of $\text{C}_{19}\text{H}_{19}\text{N}_7\text{O}_6$ with a weight (MW) of 441.4 Da. FA is very slightly soluble in water, alkaline hydroxides, and carbonates but insoluble in alcohol. Furthermore, it is a yellow or yellowish-orange crystalline compound synthetically produced and used in nutritional supplements and fortification. Folate has a typical structure characterized by a core molecule consisting of three major components, namely a heterocyclic pteridine structure, a p-aminobenzoic acid (p-ABA), and a tail of γ -glutamates. The stability decreases when exposed to or influenced by pH, temperature, light, oxidizing, alkaline media or strong acidic, oxygen, moisture, and reducing agents. When considering thermal degradation, a process more prone to occur in fortified salt, FA undergoes degradation, resulting in the formation of glutamic and pteric acid [1, 2, 3, 4, 5, 6].

FA is significant for the growth and healthy growth of babies in the womb, particularly in early pregnancy during growth. Several physiological processes have a vital role in the biosynthesis of deoxyribonucleic acid (DNA). DNA methylation plays a crucial role in cell division and tissue growth by facilitating accurate gene expression and maintaining the structure of chromosomes, as well as the prevention of neural tube defects (NTDs). Furthermore, NTDs, which include defects of the spine (spina bifida), brain (anencephaly), as well as encephalocele, are congenital malformations conditions during the structural growth of the neural tube of newborn babies. FA use

before and during the first weeks of pregnancy has been shown to reduce the risk of NTDs [7, 8, 9].

The consequences of folate deficiency can result in some birth irregularities, the chance of defects related to the brain (anencephaly), spine (spina bifida), neural tube malformations, megaloblastic anemia, neurological disorders, heart-related cardiovascular diseases (particularly strokes), specific types of cancers, cognitive impairment, and bone-related osteoporosis. This vitamin is essential for fundamental cellular processes related to amino acid metabolism, nucleotide biosynthesis, as well as methylation cycle. Unlike green plants as well as some microorganisms (microbes, fungi, some prokaryotes, certain protozoa, and bacteria), human beings do not possess the biochemical capacity to synthesize and produce folate *de novo* through similar pathways. Therefore, this natural nutrient must be acquired entirely from dietary sources [10, 11, 12, 13].

Folate includes naturally occurring derivatives in foods as well as FA, which has numerous applications in the pharmaceutical, nutraceutical, food, and beverage industries. Folate occurs naturally in plant-based food sources, including dark green leafy vegetables (DGLVs) (spinach/*Amaranthus* sp.), broccoli (*Brassica oleracea*), legumes and beans (soybean, mung bean, kidney bean), and cereals (corn). Meanwhile, FA, the synthetic form of vitamin B9, is administered primarily as an essential component in prenatal vitamins and oral dietary supplements. Additionally, it is incorporated into folic acid-fortified foods, including cereals, pasta, enriched grains, breads, breakfast cereals, grain flour, corn meals, rice, and other grain products, as well as vitamin supplements. This strategic fortification aimed to prevent folate deficiency within the general population effectively. FA is referred to as folate (Vitamin B9) when naturally occurring in food. Folate can exist in one of two forms, either the reduced, naturally occurring, or the oxidized, synthetic FA. Nevertheless, folate present in food undergoes rapid degradation, and there is limited availability of folic acid-rich foods. The artificial version of folate is more stable in oxidation and thermal processes compared to natural types. FA is the synthesized form found in fortified food and supplements, exhibiting greater bioavailability compared to naturally occurring folate. This synthetic form occurs rarely in foods or the human body. Even though folate is present in diverse foods, the intake of this vitamin B9 through the human diet may be insufficient to meet daily requirements. To address this issue, fortified foods incorporating FA have been created. However, the fortification of food with synthetic molecules may have certain drawbacks [14, 15, 16, 17, 18, 19, 20, 21].

1.2. Objective

This study was conducted to provide an overview of the principles underlying the application of the microfiltration (MF) membrane process on treating selected plant-based materials, such as DGLVs (spinach, broccoli), legume and bean (soybean, mung bean, kidney bean), and cereals (corn) to generate folic acid (FA). A series of steps included the conventional processes, enzymatic hydrolysis, nixtamalization, fermentation, separation, and purification using MF membrane. Information and data supporting this manuscript were based on many relevant and recent literature sources. Several scientific articles, literature, handbooks, and selected experimental results were also reviewed. This shows the potential uses of beneficial microorganisms producing folate as nutraceutical products for the biofortification of food.

2. DARK GREEN LEAFY VEGETABLES (DGLVS), LEGUMES AND BEANS, AND CEREALS

2.1. Dark Green Leafy Vegetables (DGLVs)

According to the dietary guidelines for Americans, it is recommended to consume five servings of vegetables per day, based on a 2000-calorie intake. Additionally, it is advised that one of these servings should consist of dark green leafy vegetables (DGLVs) [22]. DGLVs are essential to the nutrition of the residents, specifically in developing nations, because of their low prices, increased yield, a part of the local diet, and are often easily open. The most popular selected DGLVs are spinach (*Amaranthus tricolor* L.) and broccoli (*Brassica oleracea*). In Indonesia, these vegetables are termed as "Poor man's diet" because they are abundantly available all around the year at a meager price as compared to others. Generally, DGLVs are widely defined as protective foods in the human diet due to their various health advantages attributable to the rich source of vitamins (A, C, B9/folate, B12, D, K), essential minerals (Fe, Mg, K, Ca, P), fatty acids, amino acids, as well as dietary fiber, diverse bioactive compounds and an excellent

source of phytochemicals. Vitamins are vital organic molecules necessary for the normal functioning and metabolism of living cells. Each DGLV has a unique flavor, taste, and aroma, depending on the blend with other foodstuffs. These vegetables have substantial levels of FA, which has an essential function in red blood cell shape, healthy cell growth and role, and important physiological functions. A good nutrition profile is beneficial in lowering the risk of cardiovascular diseases and cancer [23, 24, 25].

Spinach (*Amaranthus* sp.) was identified as a source of folate (vitamin B9) in the late 1930s and early 1940s. Lucy Wills observed that anemia in Indian females could be alleviated with yeast extract. In 1941, this vitamin was initially separated from spinach leaves, and its name was derived from the Latin word "folium," meaning leaf. Five years later, a group of American chemists obtained a crystalline form of folate. FA is a combination of significant priority for the proper functioning of the human body, and its biologically active form, tetrahydrofolic acid, was developed in 1945 [26, 27]. Spinach is one of the essential sources of nutrients with low calories in the human diet among the DGLVs, which is regarded as a functional food (or nutraceutical) due to its diverse nutritional composition. Spinach contains high nutritional value (vitamins and minerals), as well as health-beneficial secondary metabolites, a good source of folate, antioxidants, phytochemicals, and the most nutrient-dense foods in existence promoting health aspects beyond basic nutrition [28, 29]. Spinach-derived phytochemicals and bioactive compounds can aid the prevention of osteoporosis, cut the risk of iron deficiency anemia, strengthen the immune system, assist baby growth and development, keep eyes healthy, fight free radicals, support cardiovascular health, enhance anti-inflammatory, and recover wound [30]. By virtue of its natural antioxidant properties, incorporating spinach into one's diet may help prevent various health conditions, including arthritis, cancer, cataracts, emphysema, retinopathy, atherosclerosis, cardiovascular diseases, neurodegenerative disorders, and obesity [31]. Macronutrients (per 100 gr) in spinach are calories (23 kcal), water (91.40 gr), protein (2.86 gr), carbohydrates (3.63 gr), and dietary fiber (2.2 gr). Nutritional and bioactive composition (per 100 gr) are vitamins including A (RAE, 469 µg), B1 (Thiamin, 0.078 mg), B2 (Riboflavin, 0.189 mg), B3 (Niacin, 0.724 mg), B6 (Pyridoxine, 0.195 mg), B9 (Folate, 194 µg), C (Ascorbic acid, 28.1 mg), E (Alpha-tocopherol, 2.03 mg), K (Phylloquinone, 482.9 µg), Choline (19.3 mg), and B5. Minerals consist of Mn (0.897 mg), Mg (79 mg), Fe (2.71 mg), Cu (0.13 mg), K (558 mg), Ca (99 mg), Zn (0.53 mg), P (49 mg), and Se (1 µg) [32].

Humankind has enjoyed broccoli (*Brassica oleracea* var *italica*) as a food staple for centuries. This vegetable is known to have been introduced to the rest of the world by the Italians. The name emanated from the Italian word brocco, meaning sprout or shoot. Broccoli (*Brassica oleracea* var. *italica*) is a biennial plant classified under the Brassicaceae family, characterized by its extensively separated and hunted leaves. The primary head comprises clusters of completely developed flower buds set less densely on elongated stems. Broccoli, in its sprouting forms, produces numerous small flower heads. This plant functions as an annual herb, attaining a height of 400 mm during the vegetative phase and reaching 1-2 m by the end of the flowering time. Broccoli is consumed as a vegetable throughout the world due to its nutritional benefits. Furthermore, it has played a significant role in global food techniques, serving as a source of leaf and root vegetables, fodder, as well as forage [33, 34, 35]. Beyond its culinary value, broccoli is an incredible source of many vitamins and minerals and a treasure trove of medicinal properties. Macronutrients (per 100 gr) in this plant are calories (35 kcal), water (89.25 gr), protein (2.38 gr), carbohydrates (7.18 gr), fats (0.41 gr), dietary fiber (3.3 gr), and sugars (1.39 gr). Nutritional and bioactive composition (per 100 gr) in broccoli are vitamins including A (RAE, 77 µg), B1 (Thiamin, 0.063 mg), B2 (Riboflavin, 0.123 mg), B3 (Niacin, 0.553 mg), B5 (Pantothenic acid, 0.616 mg), B6 (Pyridoxine, 0.2 mg), B9 (Folate, 108 µg), C (Ascorbic acid, 64.9 mg), E (Alpha-tocopherol, 1.45 mg), and K (Phylloquinone, 141.1 µg). Minerals consist of Mn (0.194 mg), Cu (0.061 mg), K (293 mg), P (67 mg), Mg (21 mg), Fe (0.67 mg), Zn (0.45 mg), Ca (40 mg), Se (1.6 µg), Na (41 mg), and Fluoride. Broccoli is a wealthy source of vitamins and minerals, and these compounds are responsible for its antioxidant, antimicrobial, anticancer, antidiabetic, immunomodulator, cardioprotective, hepatoprotective, and anti-amnesic properties. Broccoli has gained popularity as a favored food choice because of its abundant nutrients and fiber content [36, 37].

2.2. Legumes and Beans

Legume is a plant in the botanical family Fabaceae, also called Leguminosae, which includes leaves, stems, and

Pods. Furthermore, it is the better inclusive type for non-oil (pulse) (kidney beans, pinto beans, black beans, chickpeas, lentils, dry peas, dry beans, cowpea, faba bean) and oil-producing seed crops (peanuts, soybeans, fresh peas, fresh beans) having fresh and dried forms. The term pulse is reserved for legume crops harvested solely for the dry seed. Legumes serve as a nutritious dietary staple globally, providing an economical source of protein, vitamins, complex carbohydrates, and fiber [38]. The primary legumes utilized as food include soybeans (*Glycine soja* L.), mung beans (*Phaseolus radiatus* L.), peas, chickpeas, lentils, and peanuts. In certain regions, different terms are often used interchangeably with legumes, and the term "pulse" is commonly employed for types with low-fat content, such as beans, broad beans, peas, and lentils. Legumes are abundant sources of proteins, fats, carbohydrates, minerals, antioxidants, fiber, and water. They also serve as excellent and economical sources of β -carotene (provitamin A), thiamine (vitamin B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), folate (B9), ascorbic acid (vitamin C), as well as vitamin E and K. Additionally, they play a crucial role in providing folate in the human diet, contributing to various health benefits due to their richness in vitamins, minerals (Fe, Mg, K, Zn), dietary fiber, and diverse bioactive compounds [39, 40].

Soybean (*Glycine max* L. Merr.) is an annual plant belonging to the family Fabaceae (Leguminosae) and has its origins in the East Asia region. This vital crop exhibits adaptability to a wide range of environments, contributing to its widespread cultivation globally. Furthermore, the plant features multiple combination leaves, small white or purple flowers, and curved root pods typically housing between 1 and 4 seeds. Cultivated soybean varieties usually have a height range of 0.2-1.5 m. Due to its numerous advantages, the consumption and production of soy protein have been increased in Western countries, specifically in the United States (US) [41, 42]. Soybean ranks as the third most important food crop globally, following rice and maize. It plays a pivotal role in fulfilling the dietary and nutritional needs of a substantial portion of the world's population. Soybean stands out as one of the most valuable, versatile, and nutritionally significant legumes globally, serving as a highly effective ingredient in both food and feed. Moreover, it ranks as the second-largest source of vegetable oil. Its financial and nutritional value is attributed to a high protein range, a balanced, vital amino acid profile, and the presence of other beneficial nutrients. Soybeans are also one of the most folate-rich foods and rich in other B vitamins [43, 44]. The macronutrients (per 100 gr) are calories (122 kcal), water (69.05 gr), protein (13.09 gr), carbohydrates (9.57 gr), fat (6.7 gr), and dietary fiber (1.1 gr). Nutritional and bioactive composition (per 100 gr) are vitamins and minerals including B1 (Thiamin, 0.34 mg), B2 (Riboflavin, 0.118 mg), B3 (Niacin, 1.148 mg), B5 (Pantothenic acid, 0.929 mg), B6 (Pyridoxine, 0.118 mg), B9 (Folate, 172 μ g), C (Ascorbic acid, 15.3 mg), Cu (0.427 mg), Mn (0.0702 mg), Mg (72 mg), P (164 mg), Fe (2.1 mg), Zn (1.17 mg), K (484 mg), and Ca (67 mg) [45]. As per the Food and Agriculture Organization of the United Nations (FAO), soybean is a notable provider of both water-soluble as well as fat-soluble vitamins. It mainly boasts a rich concentration of vitamin B9 (folate), ranging from 202.90 to 450 μ g/100g. Consequently, this vegetable emerges as a promising candidate for folate biofortification to address deficiencies in the population. Soybeans are an economic origin of dietary fiber, phytonutrients, and protein significant for plant-based or flexible and vegetarian (flexitarian) nutritional patterns [46].

Mung bean (*Phaseolus radiatus* L.) is an eco-friendly food grain with multiple uses and has been described as a sustainable source of potential nutrients. The biological properties stem from the various complementary, additive, or synergistic interactions of its constituents, including high protein (20-24%), moisture (9.4%), fats (2.05%), fiber (6.4%), oil (2.1%), energy (343.5 kcal/100 g), carbohydrates, a reasonably high vitamins A and B content, and significant amounts of bioactive compounds. Among these nutrients, vitamins have gained considerable attention due to their integration into functional foods, nutraceuticals, and associated health benefits [47]. In recent years, great awareness has been concentrated on investigations into usable and value-added combinations derived from microbial seeds such as *Rhizopus oligosporus* strain-C1 (*R. oligosporus* strain-C1) and *Rhizopus* sp. for the conversion or biotransformation of mung bean to FA. This process, which occurs only in plants, bacteria, and fungi, is facilitated by the enzymes DHFS and FPGS to form dihydrofolate as well as polyglutamate folate, a result of FA [48]. The macronutrients (per 100 gr) are calories (347 kcal), water (88.26 gr), protein (28.86 gr), carbohydrates (62.62 gr), sugars (6.6 gr), fat (1.15 gr), dietary fiber (16.3 gr), and sugars (1.69 gr). Nutritional and bioactive composition (per 100 gr) are vitamins including B1 (Thiamin, 0.621 mg), B2 (Riboflavin, 0.233 mg), B3 (Niacin, 2.251 mg), B5 (Pantothenic acid, 1.91 mg), B6 (Pyridoxine, 0.382 mg), B9 (Folate, 625 μ g), C (Ascorbic acid, 4.8 mg), E (0.51 gr), and K (9 μ g). The mineral constituents comprised Ca (132 mg), Fe (6.74 mg), Mg (189 mg), Mn

(1.035 mg), P (367 mg), K (1,246 mg), and Zn (2.68 mg) [49].

2.3. Cereals (corn)

Maize, commonly known as corn (*Zea mays* L.), is a native South American word. It is extensively grown in other nations, such as Thailand, India, Pakistan, China, and several parts of the Philippines. Corn is a cereal and grain-based products annual plant with a short life cycle that belongs to the family of grass of Poaceae (Gramineae). It requires warm weather and tropical areas with well-drained and fertile soil, as well as appropriate apprehension and management. In almost every part of the world, corn constitutes the most widely distributed food plant for practical livestock feed, human food, and raw material for several industries. The quality grading of corn kernels is not only connected to the yield and quality of presentation but also has an important influence on breeding. Globally, corn is also known as the Queen of cereals due to its highest genetic gain potential among the three big staple cereals besides wheat and rice. This crop is a significant source of metabolizable energy due to a deficiency in protein (essential amino acids, lysine, tryptophan) and other nutrient content. It is also considered an important food grain in many countries of Asia, Africa, and Central and Southern America. Corn is not only the most important human food crop but has economic value and plays a diverse and dynamic role in global agri-food systems and nutrition. Corn is also a plant with a long history of medicinal uses [50, 51, 52, 53, 54, 55] and includes vitamin B-complex such as B1 (thiamine), B2 (niacin), B3 (riboflavin), B5 (pantothenic acid) as well as B6 (Pyridoxine). This vitamin is found in hair, skin, digestive, colon, heart, and brain. Corn contains vitamin B9 (folate), which prevents neural tube congenital disabilities such as spina bifida in babies. Enriched with vitamins C, A, and K, soybean is abundant in beta-carotene and contains a significant amount of selenium. This nutritional profile contributes to the enhancement of the thyroid gland and plays a vital role in supporting the proper functioning of the immune method. [56, 57]. Macronutrients (per 100 gr) in corn are calories (96 kcal), water (73.41 gr), protein (3.41 gr), carbohydrates (20.98 gr), total fats (1.5 gr), dietary fiber (2.4 gr), and sugars (4.54 gr). Corn is enriched with B-complex vitamins (per 100 gr) including B1 (Thiamin, 0.093 mg), B2 (Riboflavin, 0.057 mg), B3 (Niacin, 1.683), B5 (Pantothenic acid, 0.792 mg), B6 (Pyridoxine, 0.139 mg), B9 (Folate, 23 µg), C (Ascorbic acid, 5.5 mg), A (carotenoids) and E. The minerals in this crop include Mn (0.167 mg), P (77 mg), Mg (26 mg), Zn (0.62 mg), K (218 mg), Cu (0.049 mg), and Fe (45 mg) [58, 59, 60]. Selected plant-based foods and raw materials containing folate are visualized in Figure 1.

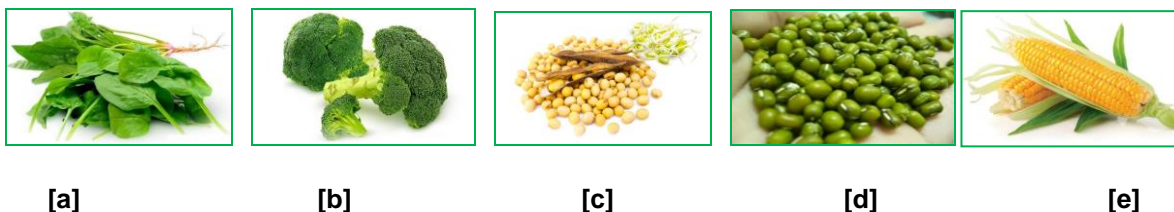


Figure 1. Selected plant-based foods raw material containing folates, such as [a] spinach [32], [b] broccoli [36], [c] soybean [45], [d] mungbean [49], and [e] corn [58].

3. THE CONVENTIONAL PROCESSES OF PRODUCING FOLIC ACID FROM BIOMASSES OF DGLVS, LEGUMES, BEANS, AND CEREALS (CORN).

Considerable attention has been focused on folic acid (FA) as one of the nutraceutical yields from the bioconversion of biomasses of DGLVs (spinach, broccoli), legumes and beans (mung bean, soybean), and cereals (corn). The conversion of these plant-based commodities into FA includes pre-treatment (breaking down the structure of raw material), enzymatic hydrolysis and nixtamalization, fermentation by microorganisms, as well as separation and purification of FA using athermal pressure-driven membrane separation methods.

3.1. Nixtamalization Process

Nixtamalization is a pre-Columbian age-old culinary process method used to soak maize (corn) dated thousands of years back to the Meso-American era. This process is one of the corn pre-treatments, besides germination, chewing or salivation, and plant material addition. Furthermore, it is a popular corn processing method commonly applied in the production of corn-based food products. Through this process, the corn kernels are cooked in an alkali solution (usually lime water) known as thermo-alkaline hydrolysis, as well as steeped in the cooking or boiling water with succeeding washing to remove any remaining organic parts and excess alkali. In the nixtamalization process, corn kernels are subjected to physicochemical transformations, manifesting in alterations to rheological, functional, and textural properties, as well as impacting the quality attributes of the ultimate food products. This process serves to soften both the pericarp and endosperm, facilitate the diffusion of water and calcium ions into the corn kernel, enhance the nutritional value and sensory properties of the food, initiate a partial gelatinization of corn starch, reduce phytate levels, improve the acceptability of the final product, and extend the shelf life of the nixtamalized products. The physical attributes of corn are crucial factors that impact both the nixtamalization process and the resulting product characteristics. Furthermore, this process can eliminate as much as 97-100% of aflatoxins from corn contaminated with mycotoxins, yielding a product commonly referred to as corn nixtamal [61, 62, 63, 64, 65].

3.2. Fermentation

The term fermentation comes from the Latin word "fermentum." The word has been extended to a natural deterioration method that includes the chemical conversion of complicated organic compounds into simpler forms by the activity of innate organic catalysts yielded by microorganisms of plant or animal sources (either inherently occurring or added) [66]. Fermentation, which is a well-known natural process used by humanity as a food processing method, can be traced back thousands of years. Furthermore, it is one of the numerous ancient and cost-effective food processing techniques. The process plays an influential role in improving and actively contributing to the nutritional value, digestibility, and sensory characteristics, including texture, flavor, and aroma. Enhancing good organoleptic properties, it elevates the quality of fermented products, promoting increased dietary value and palatability. In addition, fermentation aids in the elimination and inhibition of pathogens, toxic substances, and undesirable microorganisms, extending the shelf life and safety of food. This process not only contributes to preservation but also improves innovation, leading to the creation of new natural products. Final fermented foods usually have enhanced microbial stability and safety, and some can be stored at ambient temperatures [67, 68, 69]. Even though modern science identifies more than one kind of process, a general concept should describe fermentation as a biochemical and incomplete oxidation process via which most microorganisms convert the organic matter of complex molecules into a range of simple compound products. These compounds contain organic acids (lactic acid, acetic acid, gluconic acid, propionic acid, butyric acid, etc.), alcohols, polyphenols, and flavonoids (antioxidant, antimicrobial), vitamins (A, B-complex, C, D, E, K), minerals (Cu, Fe, Mn, Zn, etc.), CO₂ and energy [70, 71].

The fermentation-driven synthesis of vitamins employing microorganisms like fungi (yeast, molds, mushrooms), bacteria, or microbes presents several advantages over conventional chemical methods. Factors such as security, biological activity, absorption speed, and the vitamins produced through natural processes may prove more convenient for both internal and external applications [72, 73]. Fungi, namely, *Saccharomyces spp.*, *Penicillium spp.*, and *Aspergillus spp.*, are an integral component of the diverse food-making processes connected to bread, cheeses, alcoholic drinks, maceration of fruits, as well as other food applications [74]. Yeasts, classified as eukaryotic microorganisms, inhabit a diverse range of ecological niches, including water, soil, air, plant, and fruit surfaces. Among the beneficial yeasts crucial for desirable food fermentation, those belonging to the *Saccharomyces* family, specifically *S. cerevisiae*, are prominent. Yeasts contribute significantly to the food industry by producing enzymes that catalyze essential biochemical reactions, such as the fermentation of wine, beer, and ethanol, as well as the leavening of bread. In food fermentation, the

prominent bacteria include the *Lactobacillaceae* family, which can convert carbohydrates into lactic acid. Additionally, acetic acid-producing bacteria like *Acetobacter* (commonly associated with fruit and vegetable fermentation) and *Bacillus* species (often involved in legume fermentation) play significant roles. Notably, lactic acid bacteria (LAB) are pivotal in the microbial transformations observed in many commonly fermented food products [75, 76]. *Ashbya gossypii* can synthesize 0.04 mg/L of FA naturally, which can get 6.59 mg/L behind metabolic engineering therapy [77].

3.3. Separation and Purification by Microfiltration Membrane Process

The term "membrane" is emanated from the Latin word "membrana," which translates to "a skin". The membrane has been extended to describe an ultrathin flexible sheet, acting as a selective boundary barrier between two phases due to its semi-permeable properties. The layer of selective semi-permeable material with the observable thickness of the top active layer is approximately less than 1.0 μm [78, 79, 80]. Meanwhile, microfiltration (MF) is a physical separation procedure that uses porous membranes with a moderate pore size in the range of 0.1 – 10 μm . MF is selectively created to separate and remove micron-sized particles as well as other smaller solutes with linear dimensions between 0.02 and 10 μm or particles with molecular weight cut-off (MWCO) of 200,000 Dalton (Da.) from a solid/liquid suspension through a sieving mechanism based on dimensions exclusion. In its function, MF includes the use of lower Trans Membrane Pressure (TMP), which is in the scope of 0.2-5 bar [81]. The membrane, constituting the core, facilitates a flux from the feed to the permeate side when a driving force is applied, with the feed side being termed the bulk solution. The parts retained are in direct connection with the membrane. When a driving force is established, a flux occurs through the membrane from the bulk solution to the permeate side. The performance of a membrane, such as MF, is characterized by its permeability and selectivity. Membrane economics are closely linked to their transport properties, such as permeability and selectivity, which determine separation productivity and efficiency [82].

4. APPLICATION OF MEMBRANE METHOD FOR SEPARATION OF FOLIC ACID (FA) FROM SELECTED FERMENTED PLANT-BASED FOODS

Pressure-driven membrane separations, such as microfiltration (MF), are vital non-destructive methods for processing in several applications, including food and beverage, chemical, pharmaceutical, nutraceutical, and biotechnological. The application examined in the current review is restricted and selected to MF membranes and nutraceutical areas through valorization processing. This processing can be achieved through large-scale pre-treatment of biological materials, extraction or seclusion of combinations, separation of molecular fractions, purification, and incorporation into food or nutraceutical product formulation. Membrane filtration can be a valuable method for these stages to target the enrichment of distinct compounds from fermentation-based matrices with nutraceutical applications. The membranes leverage the particular chemical and physical properties of mixtures to achieve the separation of minor food components as a value-added product, all while operating at low process temperatures and minimizing energy consumption. After the development of suitable membrane materials and their long-term verification, these membrane processes have been adopted and transferred to the nutraceutical areas as a viable alternative for the treatment of folic acid (FA) and value-added compounds. Among the membrane-based separating methods that have created many options in terms of separation applications, MF is one of the most widely applied methods, specifically for the separation and recovery of FA from many selected fermented plant-based foods. These foods include dark green leafy vegetables (DGLVs) (spinach, broccoli), legumes, beans (soybean, mung bean), and cereals (corn) [83].

4.1. Fermented dark green leafy vegetables (DGLVs)

The blanching process applied to spinach and broccoli aims to achieve vegetable maturation. This process is carried out to deactivate enzymes (peroxidase and catalase), eliminate microbes, cleanse vegetables from contaminants, and eliminate undesirable flavors. The fermentation process induces a notable physical transformation, particularly in the color of the spinach suspension, shifting from fresh and pale to brownish-yellow after five days of fermentation, accompanied by a fresh scent reminiscent of vinegar, as illustrated in Figure 1.



Figure 1. (1a) Blanched spinach, (1b) suspension of blanched spinach, (1c) fermented spinach in 5 days, (1d) blanched broccoli, (1e) suspension of blanched broccoli and (1f) fermented broccoli in 5 days [83].

The outcomes of retentate and permeate resulting from the purification of fermented spinach as well as broccoli using an MF membrane with a pore size of 0.15 μm , as visualized in Figure 2.

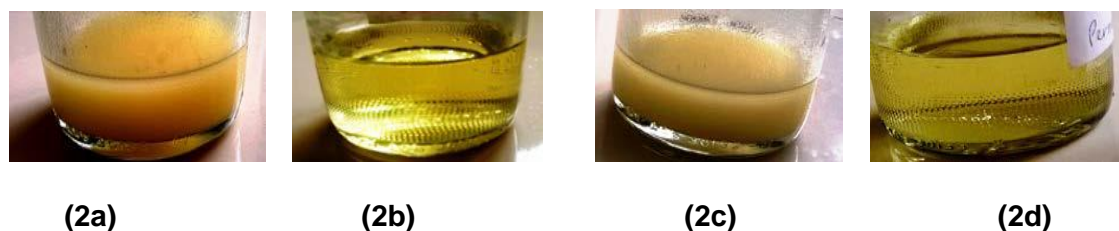


Figure 2. (2a) Fermented spinach retentate, (2b) fermented spinach permeate, (2c) fermented broccoli retentate, (2d) fermented broccoli permeate [83].

Considerable attention has been focused on FA as one of the nutraceutical yields from the bioconversion of DGLVs (spinach, broccoli). The transformation of DGLVs into FA includes a sequential process comprising the selection of fresh raw materials, blanching, fermentation using Kombucha culture, and separation through MF membrane. In the case of spinach, the production of FA follows a progression from fresh (18.16 $\mu\text{g/mL}$) to blanched spinach (14.90 $\mu\text{g/mL}$), suspension of blanched spinach (22.86 $\mu\text{g/mL}$), and fermented spinach (54.83 $\mu\text{g/mL}$). The purification of Kombucha-cultured fermented spinach through a 0.15 μm MF membrane in an SMFC at room temperature, with a stirrer rotation speed of 400 rpm and a TMP of 40 psi for 30 minutes, FA concentrations of 7.14 $\mu\text{g/mL}$ and 58.90 $\mu\text{g/mL}$ in the permeate and retentate. For broccoli, the production generated FA subsequently in fresh broccoli (21.83 $\mu\text{g/mL}$), blanched broccoli (26.85 $\mu\text{g/mL}$), suspension of blanched broccoli (30.76 $\mu\text{g/mL}$), and fermented broccoli (43.09 $\mu\text{g/mL}$). The purification of Kombucha-cultured fermented broccoli through a 0.15 μm MF membrane fitted in a SMFC at room temperature, with a stirrer rotation speed of 400 rpm and a TMP of 40 psi for 30 minutes, resulted in FA concentrations of 3.79 $\mu\text{g/mL}$ and 28.10 $\mu\text{g/mL}$ in the permeate and retentate [84].

4.2. Fermented Soybean, Mung Bean and Kidney Bean

A batch of soybeans underwent a series of steps, beginning with sorting to select uniform and high-quality beans. These selected beans were then cleaned, blanched for 30-45 minutes, cooled to space temperature, as well as soaked in water overnight at pH 5. Subsequently, the soybeans were hulled, cleaned, and inoculated with a 0.2% (w/w) inoculum of *Rhizopus oligosporus* C1 at 30 °C for 72 hours. Concurrently, mung and kidney beans were subjected to incubation with a mixture of *Rhizopus* inoculum (0.2%, w/w) at 30°C for 72 hours. To facilitate the uniform growth of *Rhizopus* inoculum, three (3) fermented beans were wrapped with a perforated plastic sheet, effectively reducing the evaporation rate during the tempeh production process. A part of soybean tempeh, mung bean, as well as kidney bean was mixed with distilled water (1:1, w/v), pulverized, filtered through a 100 mesh sieve, and diluted to 1:2 (w/v). Furthermore, the beans' tempeh filtrate obtained was diluted by adding 1 part of water to form a suspension from soybean, mung bean, and kidney bean. The composition of soybean tempeh suspension is total solids, dissolved protein, N-amino, and FA of 5.70 %, 0.78 %, 3.78 mg/mL, and 606.74 $\mu\text{g/mL}$, respectively. The composition of mung beans tempeh suspension is total solids of 3.87 %, dissolved protein of 0.75 %, N-amino of 2.24 mg/mL, and FA of 242.93 $\mu\text{g/mL}$. The composition of kidney bean tempeh suspension is total solids, dissolved protein, N-amino, and FA of 1.33 %, 0.76 %, 0.42 mg/mL, and 354.71 $\mu\text{g/mL}$.

The suspension underwent further purification by passing through a 0.15 μm MF membrane, implemented in dead-end stirred filtration cell (DESF) mode. Operating conditions included room temperature, a stirrer rotation speed of 400 rpm, and a TMP of 40 psia for 30 minutes. Samples of permeate were collected and recorded to determine the flux through the membrane. Regular sampling of permeate and retentate was conducted for subsequent analysis. Folate (FA) concentrations in the permeate of soybeans, mung beans, and kidney beans tempeh suspension were found to be 197, 325.37, and 242.93 $\mu\text{g/mL}$, respectively, while the content in the retentate was 362.07, 254.07, and 506.07 $\mu\text{g/mL}$. The interaction between various types of tempeh and membrane performance significantly influenced the separation.

The majority of components in the tempeh protein isolate were retained in the retentate, with a notable difference compared to those passing through into the permeate. Microfiltration successfully recovered soybean, mung bean, and kidney bean tempeh isolates, containing FA concentrations of 64.76%, 43.85%, and 67.57%, total solids of 80%, 61.8%, and 79%, N-amino of 78%, 50%, and 66.7%, and dissolved protein of 71.8%, 54%, and 64%, respectively. The retentate from soybean, mung bean, and kidney bean tempeh protein isolates contained FA concentrations of 362.07, 254.07, and 506.07 $\mu\text{g/mL}$, total solids of 5.56%, 4.08%, and 1.82%, N-amino of 4.34 mg/mL, 3.36 mg/mL, and 0.56 mg/mL, as well as dissolved protein of 0.79 mg/mL, 0.34 mg/mL, and 0.72 mg/mL, respectively. Under these conditions, the MF membrane exhibited the ability to increase FA in retentates from soybeans by 59.67% (0.59-fold), mung beans by 110.58% (1.1-fold), and kidney beans by 142.67% (1.42-fold) before the purification process. Figure 3 depicts the feed, retentate, and permeate resulting from the separation and purification of FA from soybean tempeh suspension using the MF (pore size 0.15 μm) membrane [85].

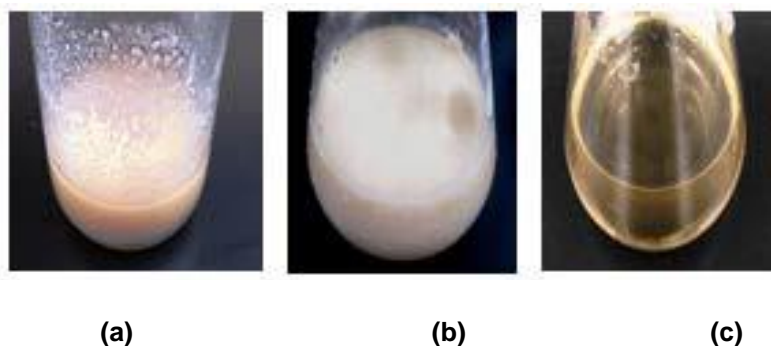


Figure 3. (a) feed, (b) retentate and (c) permeate as a result of separation and/or purification of folic acid from soybeans tempeh suspension by MF (0.15 μm) membrane [85].

4.3. Fermented Corn Nixtamal

Horse dent corn (*Zea mays* var. *indentata*) is a corn variety cultivated commercially for human consumption, offering various functionalities. Despite its relatively uncommon presence, corn surprisingly contains a substantial protein content, ranging from 7-10%. The diversification of corn as a natural source of folate (Vitamin B9) involves chemical modification (nixtamalization) and enzymatic processes (fermentation) to recover folate as a fortifying agent. Any alteration in the nixtamalization of corn, especially the selection of starter cultures, has the potential to influence the sensory properties of the final product. Moreover, the process of fermentation can significantly impact the organoleptic characteristics of corn nixtamal. One application of a pressure-driven membrane system, specifically Microfiltration (MF), involves separating and purifying folate and valuable components produced through a series of processes by *Rhizopus oligosporus*-C1 as broth. In the MF mode, a low-pressure broth solution, based on molecular size, is pushed against a membrane with pores sized at 0.15 μm , corresponding to the molecular weight (MW) for separation. The relatively high MW parts are retained entirely on the membrane's top surface, referred to as non-permeable solids retentate (concentrate), utilizing a sieving mechanism [86, 87]. Folic acid content in the kernel of yellow horse dent corn (YHDC), corn nixtamal, corn nixtamal fermented by *R. oligosporus* strain-C₁ (CNF), feed of CNF, retentate of CNF, and permeate of CNF is 95.83, 179.12, 123.31, 206.40, 212.84, and 121.10 $\mu\text{g/mL}$, respectively. Figure 4 shows the Kernel of YHDC, corn nixtamal, inoculum of *R. oligosporus*

strain-C₁, corn nixtamal fermented by *R. oligosporus* strain-C₁, feed of CNF, retentate of CNF, and permeate of CNF [86].

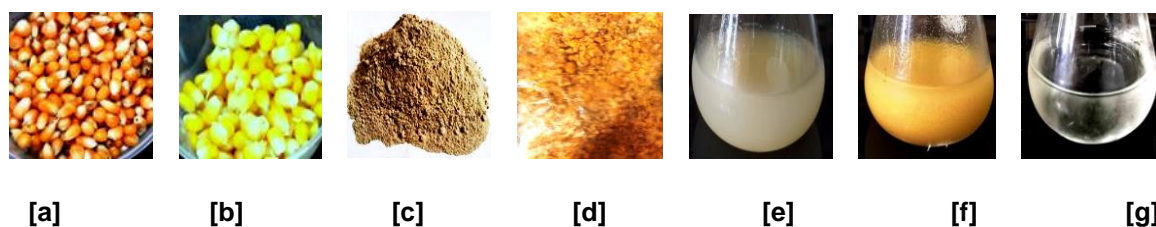


Figure 4. (a) kernel of yellow horse dent corn (YHDC), (b) corn nixtamal, (c) inoculum of *R. oligosporus* strain-C₁, (d) corn nixtamal fermented by *R. oligosporus* strain-C₁ (CNF) (e) feed of CNF (f) retentate of CNF, and (g) permeate of CNF [86].

CONCLUSION

In conclusion, DGLVs, legumes, beans, and corn were considered the cheapest sources of vegetables and described as poor man's food despite their vitamins, minerals, and micronutrient content. The consumption could improve nutritional status, repair body tissues, and reduce risks of specific diseases such as diabetes and cancer. DGLVs, legumes, beans, and corn were significant plant-based food sources for the world's population and represented the vehicle for vitamin and mineral deficiency intervention. MF was one of the contemporary pressure-driven membrane processes that had become viable athermal separation unit operations in chemical engineering. The principal benefits of MF membrane processes compared to other separation processes were non-thermal, no phase change, low energy consumption, separation of components in the native state (heat-sensitive products), simplicity in operation and maintenance, less production of sludge, and environmental friendliness. The recovery of folic acid (FA) allowed the valorization of the co-product in the nutraceutical field and mitigated the environmental damage. This review opened the way for the separation and purification of folate from plants-based food fermentation broth with MF membrane technology results as a reference for future study.

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