Chapter

Functional-Antioxidant Food

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Abstract

Nowadays, people face many different dangers, such as stress, unsafety food, and environmental pollution, but not everyone suffers. Meanwhile, free radicals are the biggest threat for humans because they lead to over 80 different diseases composed of aging. Free radicals can only be eliminated or minimized with antioxidant foods or antioxidants. The chapter on the functional-antioxidant food presents the antioxidant functional food concept, the classification, the structure, and the extraction process of antioxidant ingredients. Various antioxidant substances such as protein (collagen), polysaccharides (fucoidans, alginates, glucosamines, inulins, laminarins, ulvans, and pectins), and secondary metabolites (polyphenols (phlorotannins, lignins, polyphenols), alkaloids, and flavonoids) also present. The production technology, the mechanism, the opportunity, and the challenge of antioxidants functional food also present in the current chapter. The current chapter also gives the production process of functional-antioxidant food composed of the capsule, the tablet, tube, the pills, the powder, and the effervescent tablet.

Keywords: antioxidant, functional food, proteins, secondary metabolites, polysaccharides

1. Introduction

Today, the greatest danger of humans is free radicals, the source of about 80 different human diseases including aging [1–14]. Free radicals are produced from the pollution of the climate environment, water, food, human life, and work, and also from the natural transformation of the earth [8, 15]. In order to reduce free radicals, nature itself also has complex metabolic processes to produce inactivates and convert free radicals to a more stable form in nature. Free radicals are known as products of antioxidants that occur naturally or are produced by biosynthesis [1, 7]. These antioxidants are mainly in the form of biopolymers such as proteins, polysaccharides, and secondary metabolites (polyphenols, alkaloids, flavonoids). Each group of antioxidants possesses different antioxidant properties for different applications. They are found in a loose or persistent bond in natural resources and are difficult to extract in varying concentrations into different species. Under the increasing pressure of society and nature, human aging and disease are increasing [4, 9]. To meet the social needs

and personal development. Antioxidant supplements are increasingly popular and welcomed by consumers, and are more interested in by regulators, manufacturers, and researchers.

Hence, the chapter focus on the functional-antioxidant food composing of the structure, the extraction process, and the production technology of antioxidants into functional food, the mechanism of functional-antioxidant food. Functional-antioxidant food will mainly contain protein, polysaccharides, and secondary metabolites, for example, polyphenols (phlorotannins, lignins, polyphenols), alkaloids, and flavonoids. Antioxidant polysaccharides focus on fucoidans, alginates, glucosamines, inulins, laminarins, ulvans, and pectins, while protein is collagen from marine resources. Antioxidant functional foods will exist in the capsule, the tablet, tube, and the effervescent tablet. The materials used for extracting bioactive substances are diverse from terrestrial flora and fauna to the sea.

2. Functional-antioxidant food - concept and classification

2.1 Concept

Functional-antioxidant food is food containing one or more antioxidant substances extracted from plants or animals or synthesized. Bioactive substances belong to the group of protein, carbohydrate, or secondary metabolites.

2.2 Classification

Functional-antioxidant food could be classified according to different criteria, for example, shape or bioactive ingredient style. Classification of functional-antioxidant food follows their shape are susceptible to drug classifications. Hence, functional-antioxidant food classification base on their bioactive ingredient style (**Figure 1**). Functional-antioxidant food is mainly three large groups (protein, carbohydrates, and secondary metabolites. The current chapter presents antioxidant substances commonly used in the manufacture of functional-antioxidant food such as such as collagen, pectin, alginate, fucoidan, laminarin, inulin, glucosamine, ulvan, chondroitin, polyphenol, lignin, alkaloid, and flavonoid.

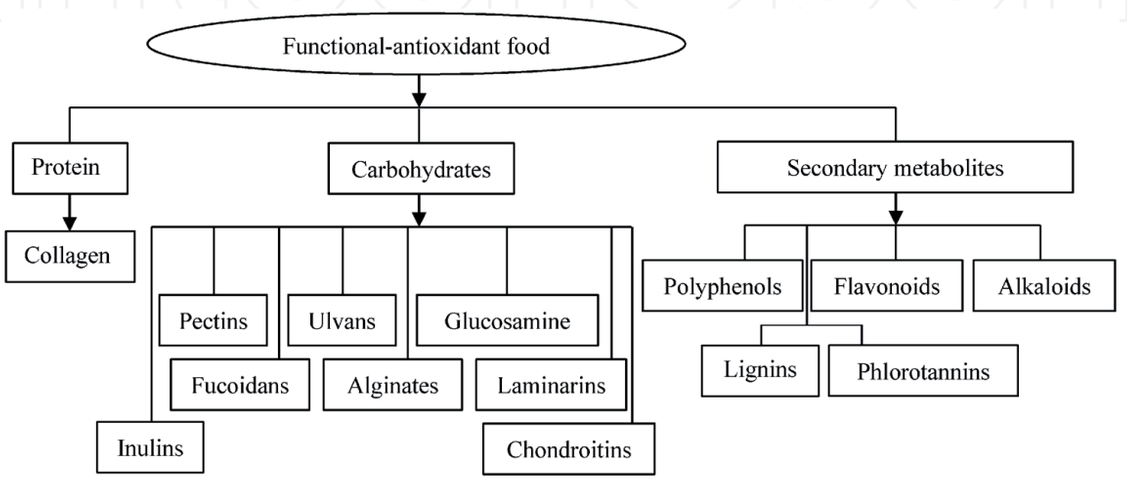

Figure 1.
Classification of functional-antioxidant food.

3. Antioxidants in nature

3.1 Protein (colagens)

Collagen belongs to the endogenous protein group, is found in connective tissues of vertebrates (skin, scales, bones, articular cartilage, blood vessels, and tendons), and contains basal components consisting of glycine, proline, alanine, and hydroxyproline. Glycine plays as the helical center in the structure of collagen. Collagen possesses a molecular weight of about 100,000 daltons, crosses intermolecular bonds, and the repeated glycine-proline-hydroxyproline chains [16]. Tiago *et al.* noticed that the scientists identify at least 28 collagen types, but the main of types I (bones, skin, tendons, and organs), II (cartilage), and III (reticular fibers, blood, and skin). The most prevalent invertebrates contain I to IV types collagens [17]. The structural stability of collagen depends mainly on the ratio of glycine and hydroxyproline that is different between animal species, even in a species. Various structural, chemicals, and amino acid content in glycoproteins lead to the difference. The collagen of fish is similar to mammalian on amino acid composition. Collagen having a molecular weight up to 3–10 kDa possesses antioxidant activity higher than other fractions. The antioxidant activity of collagen in fish is higher than that one in animals [18]. Differences in the structure and the molecular weight of acid-soluble collagens extracting from rainbow trout skins that have grown in the sea and freshwater do not occur. In rainbow trout skins, glycine content is the highest, following alanine, proline, and hydroxyproline with the various chains such as α_1-, α_2-, and β [19]. Denaturation temperature of fish collagen (25–30°C) is low in comparison to mammalian collagen (39–40°C) [20].

3.2 Polysaccharides

3.2.1 Fucoidans

Fucoidans are only found in brown algae, belong to the sulfated anion polysaccharide group. L-fucose is a basic unit in fucoidans. The primary linkage of (1 → 3)-α-L-fucopyranosyl and the linkage of alternating α(1 → 3) and α(1 → 4)-L-fucopyranosyls occur in fucoidans structure [21, 22]. Fucoidans are structurally diverse and different between various brown algae species, even in a season that leads to the difference in their content. Therefore, fucoidans exhibit bioactive diversity, for example, antioxidant [21, 23], antitumor, antibacterial, anticoagulant, anticancer [22], antivirus, immune activation, neuroprotective, and the protection of the stomach and liver [24]. Fucoidan contents in sterile tissue (dry matter) and reproductive tissue of kelp species ranged from 0.5–13% and 1.4–69%, respectively [25]. For brown algae grown in Vietnam, fucoidan content usually ranges from 0.8 to 3.5%, compared to dried algae. Fucoidans that extract from brown algae have a color range from light brown to dark brown, depend on their purification.

3.2.2 Alginates

Alginates belongs to sulfate polysaccharide, exist in brown algae species with the linear structure of copolymers, and the basic units of β-D-mannuronate (M) and α-L-guluronate (G) that links via (1,4)-glucoside linkage. Alginate content is about 15 to 25% of dry algae [26]. Nowadays, over 200 different alginates appear in the market [27]. Different alginates have various G/M ratio and molecular weight that depend on the season, species, and growth sites. G/M ratio and molecular weight play an important role in exhibiting bioactive and application of alginate. Alginates form a

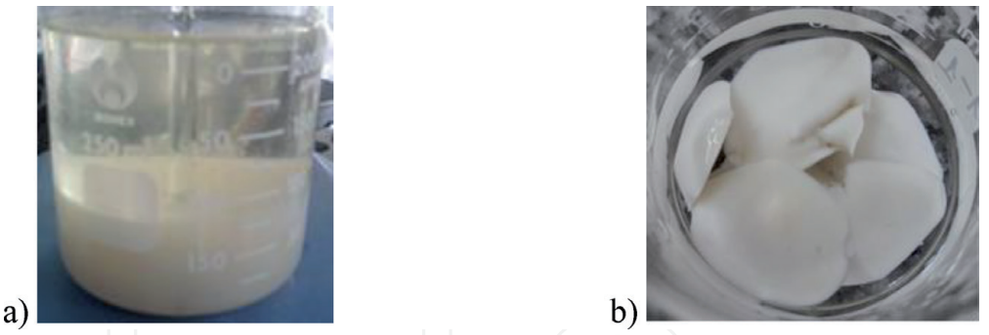

Figure 2.
(a) Alginate in 96% ethanol; (b) pectin from cactus Opuntia dillenii.

robust and rigid gel when low the M/G proportion and large guluronic blocks ratio in their structure, and reverse forming soft and elastic gels [28, 29]. Alginates possess a molecular mass larger than 50 kDa exhibiting the prevention ability of diabetes and adiposity. Numerous studies showed that antioxidant activity is also one of the bioactive characteristics of alginates [30, 31]. Therefore, alginates are useful in the food, cosmetic, functional food, pharmaceutical, even dentistry, and toothpaste such as stabilizers, emulsifying agents, or thickeners. Puried alginate has white color (**Figure 2a**).

3.2.3 Glucosamine

Glucosamine sulfate (2-amino-2-deoxy-D-glucose) is the sulfate derivatives of chitosan that formed after deacetylation for chitin. Glucosamine sulfate is an essential amino monosaccharide in connective tissues (cartilage and ligaments) of marine invertebrates and commonly the material for synthesizing glucosaminoglycans, glucoprotein, and glucolipide [32]. Nowadays, crab, lobster, or shrimp shells are materials for producing glucosamine. Numerous studies showed that glucosamine could exist in glucosamine hydrochloride, glucosamine sulfate, and N-acetylglucosamine and links to chondroitin sulfate in the connective tissues. The biological activity of glucosamine is proven (antioxidant, antiinflammatory, induce ER stress, antigenotoxic, cardioprotective, neuroprotective, O-GlcNAc modification, and antifibrotic) and is in positive proportion to the sulfate groups in their structure [33, 34].

3.2.4 Inulins

Inulins belong to the fructooligosaccharides group, composed of linear fructosyl polymers and oligomers with degree polymerization (DP) (3–65). DP of inulins in chicory consists is from two to approximately sixty units. In inulin, terminal glucose residues unit the non-reducing end via an α-(1,2) glycosidic bond and contains two or more fructosyl moieties that link each other by β-(2,1) bonds. The fructooligosaccharides (fructose oligomers) possess one glucose unit and two to four fructose units. Short fructooligosaccharides compose of 1-kestose, nystose, and 1F-fructofuranosylnystose. Small inulin oligomers (degree polymerization <10) are oligofructose and fructooligosaccharides. Inulins in plants and fungi contain β-(2,1)-D fructofuranosyl units [35]. Antioxidant activity of inulin is higher than simple sugars (fructose, glucose, and sucrose) and stable under the impact of the cooking and digestion processes (pH changes, digestive enzymes). Inulin unaltered better than ascorbic acid that lost from 40 to 90% of antioxidant activity at high temperatures. The antioxidant role of inulins exhibit better than other ROS (radical oxygen system) scavengers (vitamins C and E – the absorbance in the first part of the gut) because inulin is absorbent in the colon that occurs in vitamins C and E.

Inulins against protein oxidation are basing on the protection of the mucosal and the submucosal layers. Fructans (inulin style) respond to a defensive role against oxidative stress, at the same time activating automatic before the endogenous systems of detoxification in rats. Radical oxygen system scavenging capability of oligosaccharides in intra-peritoneal administration in vivo decide the decrease of lipid peroxidation. Besides inulins, levans (high molecular weight polymer) are about 107 Da with type β-2,6 linkages. Galactopyranosyl oligomers possess DP (3–8) with mostly β-(1,4) or β-(1,6) bonds and less β (1,2) or β-(1,3) linkages. Levans can combine various metal nanoparticles such as Levan–Fe^{2+} and levan–Cu^{+} that ROS inhibition up to 88% and 95%, and the combination exhibit antioxidant activity better than 33–40%, compared to single levans [35]. Moreover, levans possess numerous bioactivities, for example, antioxidants, anti-tumor, and anti-inflammatory [36].

3.2.5 Laminarins

Laminarins are found in brown algae, belong to a linear sulfated polysaccharide that soluble in water and 22–49% of the dry algal mass. Laminarins are known as a β sulfated glucan consists of a 3:1 ratio of β (1 → 3) and β (1 → 6) with a molecular weight of 5 kDa and (1 → 3)-β-d-glucopyranose residues [37]. Laminarin structure depends on algae species, growth period, and growth condition, while molecular weight is affected by the polymerization degree. Laminarin could be M chains or G chains, corresponding to the terminal 1-O-substituted D-mannitol or glucose, respectively, depends on the sugar type at the reducing end of laminarin [38]. Numerous studies showed that laminarins as a potential bioactive ingredient in cancer treatment basing on antioxidant activity and inhibition (melanoma cells, colon cancer, and anti-metastatic). The structure, molecular weight, monosaccharide unit number, degree polymerization, and branching length of laminarin control their antioxidant activity [39, 40]. For example, laminarins (15 and 06 kDa) possess antioxidant activity (7.5 and 79.7%), respectively. DPPH scavenging activity of purified laminarin (10 kDa) corresponds to 87.57%. The antioxidant activity of laminarin is basing on the interaction between carbonyl groups and transition metal ions (Cu^{2+} or Fe^{2+}) and carbonyl groups [39]. Laminarins also exhibit the ability to the inhibition of lipid peroxidation. Laminarins also play a role in the inhibition of chain initiation, peroxide decomposition, and binding of transition metal ions.

3.2.6 Ulvans

Ulvans are mainly high sulfated polysaccharide existing in green algae (Ulva and Enteromorpha) and animal (glycosaminoglycans), belongs to heteropolysaccharides group, and water-soluble. Ulvans contain the basic units such as xylose, rhamnose 3-sulfate, iduronic acid, xylose 2-sulfate, and glucuronic acid and account for about 38 to 54% of dry algae mass. The units of α-L-rhamnose-3-sulfate-1,4-β-D-glucuronic acid, α-L-rhamnose-3-sulfate-1,4-α-D-iduronic, and α-L-Rhamnose-3-sulfate-1,4-β-D-xylose are repetition in the structure of ulvans [41]. Ulvans and their-derived oligosaccharides exhibit antioxidant activity via the level decrease of the total and LDL cholesterol and triglyceride reduction in the serum. The molecular weight of ulvans is a positive proportion to their antioxidant capacity. For example, hydroxyl radical scavenging ability and the molecular weight of ulvans ranged from approximately 50 to 90% and 18.2 to 100.5 kDa, respectively. Sulfate group number in ulvans also affect their antioxidant activity. For example, 2.0 mg/mL of ulvan (32.8% w/w sulfate) of *U. pertusa* species arrested 90% hydroxyl radical that higher than native ulvan possessing 19.5% w/w sulfate [42]. Therefore, the over-sulfation of ulvans will have more benefits for antioxidant activity.

3.2.7 Pectins

Pectins exist mainly in cell walls of terrestrial and marine plants, classified into a heterogeneous polysaccharide group. Pectins contain over 65% of 1,4-linked-α-D-galacturonic acid that depends on species, for example, the galacturonic acid content of pectin in mangosteen rind (73.16%), in lime (72.5%), and mango peels (56.67%) [43]. Pectins are diverse on the structure and is usually classified based on esterified galacturonic acid units (methoxylation degree) as well as possesses different biological activities (antioxidant, antitumor, and anti-inflammatory). The main pectins in a plant are homogalacturonan, and unpopular (xylogalacturonan, rhamnogalacturonan I, rhamnogalacturonan II, arabinogalactan I, and arabinogalactan II. The structure, the content, molecular weight, and the plant species lead to a difference in the reaction rate constant between pectins and hydroxyl radical scavenging. Pectins in fresh white cabbage, carrot, onion, and sweet pepper exhibit antioxidant activity lower than Opuntia ficus indica. The rating constant for the reaction range from $2.05 \pm 0.56 \times 10^9\ M^{-1}\ s^{-1}$ to $(1.03–1.37) \times 10^9\ M^{-1}\ s^{-1}$. The rate constant also depends on the compositions in pectin compounds such as protein or secondary metabolites. Xanthine oxidase inhibition of pectin is improved when the molecular weight and a Gal residues number in pectin structure are high. Pectin of onion control inhibition of xanthine oxidase better than radical scavenging of DPPH and the hydroxyl [44]. Low etherified pectin helps the stabilization of malonic dialdehyde level and the inhibition of glutathione reductase and glutathione peroxidase [45]. Pectins have the content (2–35%) and the molecular weight (25–360 kDa) depending on plant species. Pectin after purifying is also white color, same purified alginate (**Figure 2b**).

3.3 Secondary metabolites

3.3.1 Polyphenols (phlorotannins, lignins, polyphenols)

Polyphenols are diverse in structure and exist in all different plants as well as marine (sponges). Polyphenol is named phlorotannin in marine. For terrestrial plants, polyphenols are determined, such as quercetin, tannin, gallic acid, mangrin, resveratrol, and lignin. Functional-antioxidant food is commercial in the market, for example, tannins, quercetin, gallic acid, mangrin, phlorotannins, or resveratrol, but not lignins. Lignins are antioxidant polyphenols existing in all plants interesting in the near time, especially their application into functional food and pharmaceuticals. Therefore, phlorotannins, lignins, and polyphenols are focused on the current chapter (**Figure 3**).

Phlorotannins that compose of the phloroglucinol units and the various linkages (ether, phenyl, ether/phenyl, and dibenzodioxin) are polyphenols in all marine plants and some marine animals [46]. Therefore, the structure of phlorotannins is diverse, and the thing leads to other bioactive of them, for example, antioxidant

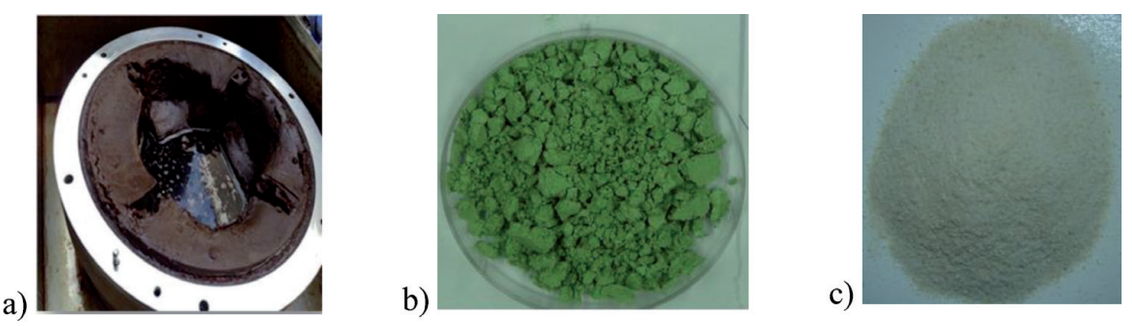

Figure 3.
(a) Crude fucoidan from brown algae Sargassum duplicatum grown in Vietnam; (b) chlorophyll powder from maize leaves; (c) glucosamine from shrimp shell.

[31, 47, 48], antitumor, anticancer, and inhibition of UV radiation [49]. Phlorotannins have two styles of free (existing in membrane-bound vesicles) and cell wall linkage (phlorotannins-alginic acid). Their content in brown algae is more than different marine organisms [50]. Phlorotannins content could reach up to 2% in brown algae growth in temperate regions of Pacific and Atlantic and tropical Atlantic regions [51]. Some studies noticed that phlorotannins in thallus dry weight are up to 25–30% [52]. For brown algae grown in Vietnam, antioxidant phlorotannin content is from 0.1–1.1%, compared to dried algae.

Lignins exist in the matrix of hemicellulose, cellulose, and lignin in the cell wall. Lignins are classified into an irregular polyphenol with monolignols (cinnamyl alcohols (guaiacyl), coniferyl alcohol (syringyl), sinapyl alcohol (p-hydroxyphenyl), and p-coumaryl alcohol) that cross-linked together via the linkages of carbon–carbon, ester, and ether. The ratio of monolignols in lignin structure is different between various plants [53]. Dehydrogenative polymerization of phenyl propanoid units helps to the synthesis of irregular lignin more advantageous. In-plant, lignins are formed via the metabolic pathway of phenylalanine/tyrosine. They account for up 10 to 25% of the dry plant mass and 1 to 43% in lignocellulosic biomass (cellulose, hemicellulose, and lignin). In the sugarcane and corn (stalk, stover, and straw), lignins are up to 25–32% and (6.9, 19.54, and 7.5), respectively [54]. Lignins are known for the free radical scavenging activity (2,2-diphenyl-1-picrylhydrazyl, DPPH• and (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid, ABTS). The antioxidant efficiency of lignins depends on the structure and the solubility of lignins. Antioxidant activity is affected by the carbonyl groups of side chains but free hydroxyl groups and ortho-methoxy substitution in phenol ring impact. The molecular weight, the polydispersity, and the heterogeneity of lignins play a role in controlling their free radical scavenging capacity [55].

Polyphenols are known with one or more hydroxyl groups on the aromatic skeleton ring and possess antioxidant activity (scavenge free radicals, and inactivate prooxidants) [56], heart disease prevention, inflammation-reducing, anti-cancers, and antidiabetic, as well as the rate reduction of mutagenesis in human cells. Polyphenols are different in the structure, content, and antioxidant activity in various plants [57]. Nowadays, based on chemistry characteristics such as chemical structure, simple molecules, and highly polymerized compounds, scientists classify about ten different classes with over 8000 polyphenol structures. The relationship between antioxidant activities and the chemical properties of polyphenol is also noticed and demonstrated very clearly. Polyphenols presented in the current section are free polyphenols and belong to the group that dissolves in an organic solvent and aqueous, not alkaline and acid [58]. Common polyphenols in terrestrial plants are known, such as quercetin, rutin, tannin, gallic acid, catechin, resveratrol, mangiferin, and epicatechin.

3.3.2 Alkaloids

Alkaloids are one of phytochemistry composition in plants, composed of at least one nitrogen atom with hydrogen-carbon groups in an amine-type structure, and accumulate nearly 20% of plant species. They are mainly well-known as pyrrolizidines, pyrrolidines, pyridines, isoquinolines, tropanes, indoles, quinolines, morphine, strychnine, quinine, ephedrine, and nicotine [59]. Alkaloids possess antioxidant activity, such as radical scavenging potential, total antioxidant activity, ferric reducing antioxidant potential, hydroxyl group scavenging ability, and lipid peroxidation inhibition ability. Alkaloids play a controlling role in antioxidant activity better than phenols [60, 61]. Alkaloids content and their activity are a correlation to the species and growth time of plants. For example, alkaloids are the major antioxidants in maca. Hydroxylated alkaloids exhibit antioxidant activity based on the reaction between radicals with high lipophilicity. However, the solvation process causes a decrease in the antioxidant activity of alkaloids [62].

3.3.3 Flavonoids

Flavonoids are secondary metabolisms belonging to the polyphenol group, consist of two phenyl rings and a heterocyclic ring, and abbreviated C_6-C_3-C_6. Flavonoids are commonly flavones, flavonols, flavanones, flavanonols, flavanols or catechins, anthocyanins, and chalcones [63, 64]. Flavonoids exist in almost various plants and possess high bioactivities in *Morinda* L. [65]. Flavonoids in *Morinda* are antioxidants having the ability against cardiovascular disease and cell components degeneration that age-related [66]. The mainly antioxidant properties of flavonoids are based on a role against free radicals (hydroxyl and superoxide radicals) via scavenging of reactive species and inhibiting biomolecular damage [67]. Besides, flavonoids also exhibit various bioactivities, such as anti-diabetic, anti-inflammatory, cardioprotective activity, anti-age-dependent-neuropathology activity, anti-cancer activity, and anti-viral/bacterial [68]. Flavonoids content corresponds 5 to 10%, compared to the secondary metabolites in plants, and nowadays, there are about 5000 identified flavonoids. Some studies presented an intake dose of flavonoids per day is from 20 mg and 500 mg [69].

4. Extraction process

Nowadays, there are numerous extraction methods of bioactive substances from nature for the application into functional food and pharmaceuticals. For example, maceration, reflux, soxhlet, microwave-assisted, ultrasonic-assisted, enzyme-assisted, and gamma Coban 60-assisted. Different bioactive ingredients will have specifically suitable extraction methods, and the **Figure 4** will present the thing.

4.1 Collagen

The high temperature leads to the degradation of collagen structure, so they are usually pre-treatment, extracted, and purified under cold temperatures (≤ 5°C). After pre-treatment, skins and scales will be dried by using infrared – assisted

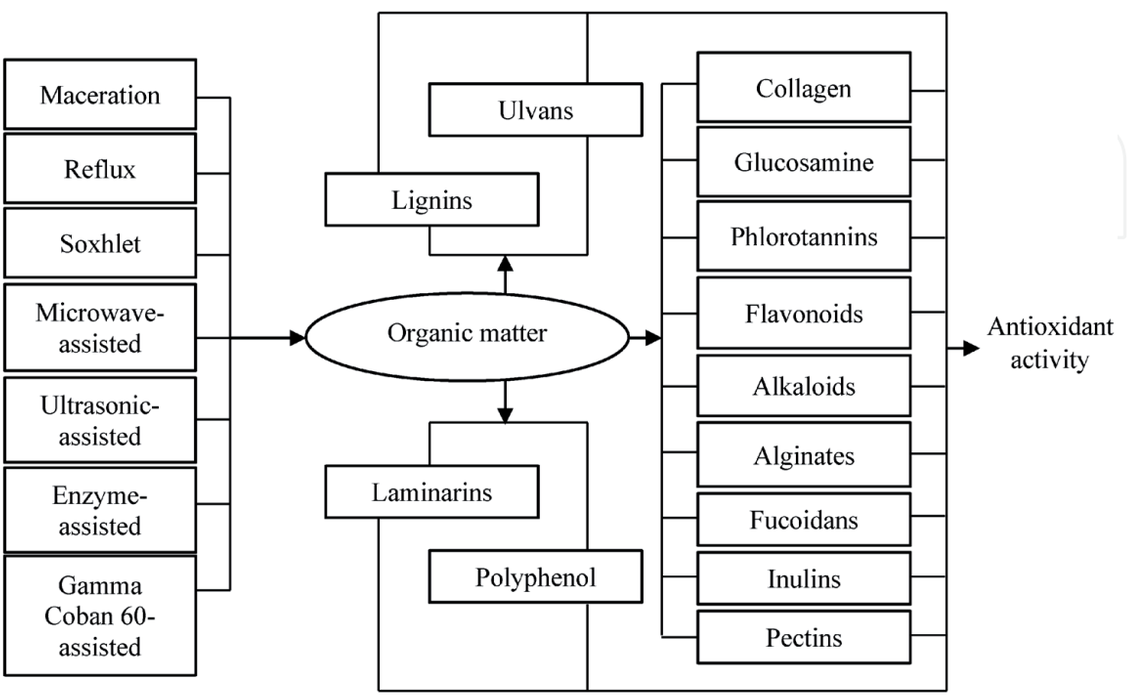

Figure 4.
General extraction schematic of antioxidant ingredients from organic materials.

cold drying and minced. For collagen extraction, the solvent of CH_3COOH is usually useful (**Figures 5** and 6). For fish skins, removing non-protein and lipid are by using 0.1 M NaOH and 10% butyl alcohol, respectively (**Figure 5**). NaCl and ethanol are suitable for the precipitation of collagen from skins and scales, respectively.

Fish skin is scraped, washed to remove impurities, washed with cold water to remove impurities, then chopped with a size of 0.5 x 0.5 cm. After soaking for enough time, the fish skin is removed and rinsed with cold water until neutral pH. Fish skin will be extracted with 0.5 M CH_3COOH solution at 1/15 (w/v) ratio for 48 hours and precipitated in 2.6 M NaCl in 0.05 M tris buffer (hydroxymethyl) aminomethane (pH 7.0) (**Figure 6**). The precipitate was then centrifuged at 20,000 g for 60 minutes at 4°C, then undergone dialysis and finally lyophilized to collect the collagen. The efficiency obtained from the bovine skin with acetic acid

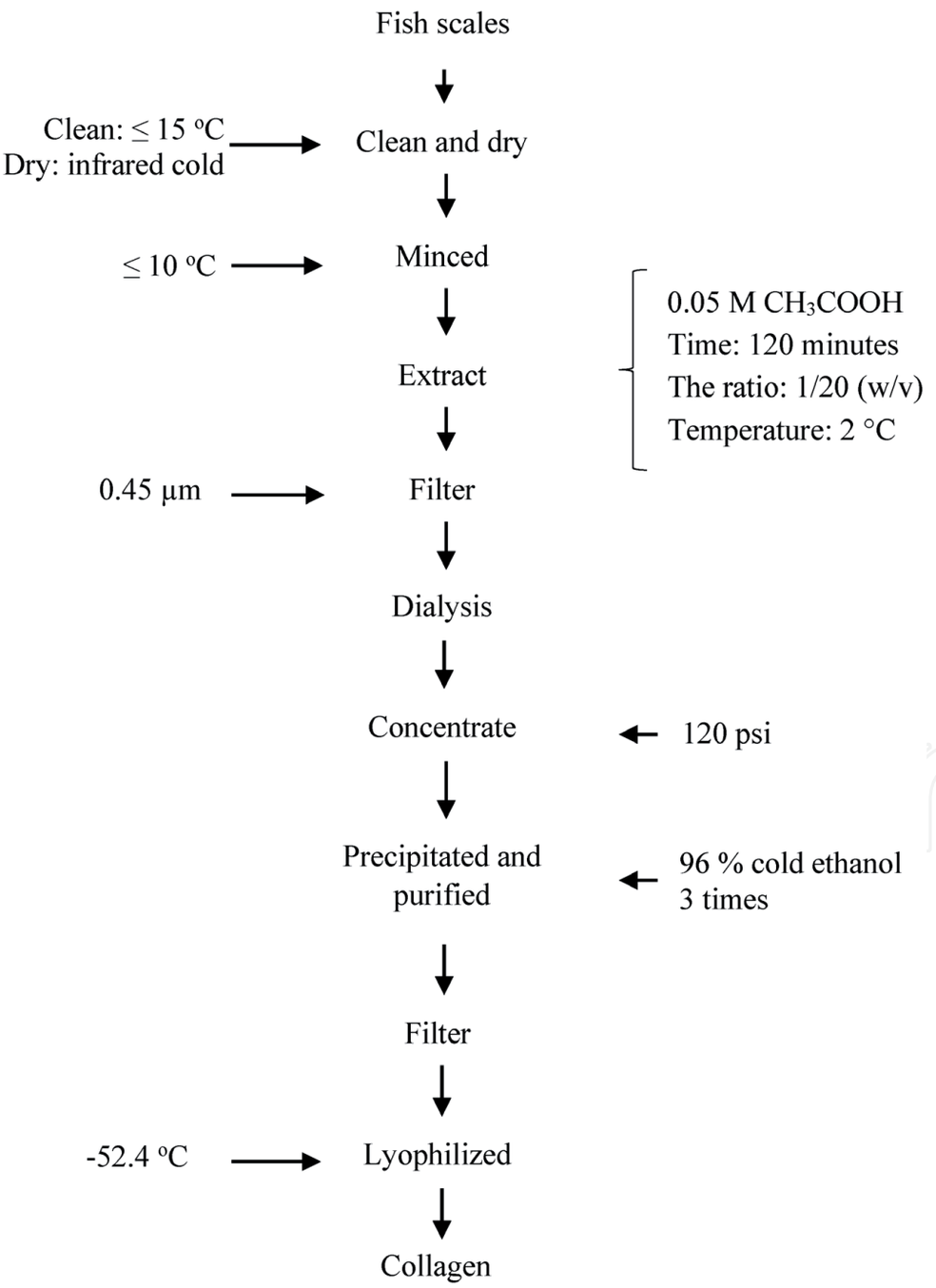

Figure 5.
Extraction schematic of antioxidant collagen from fish scales.

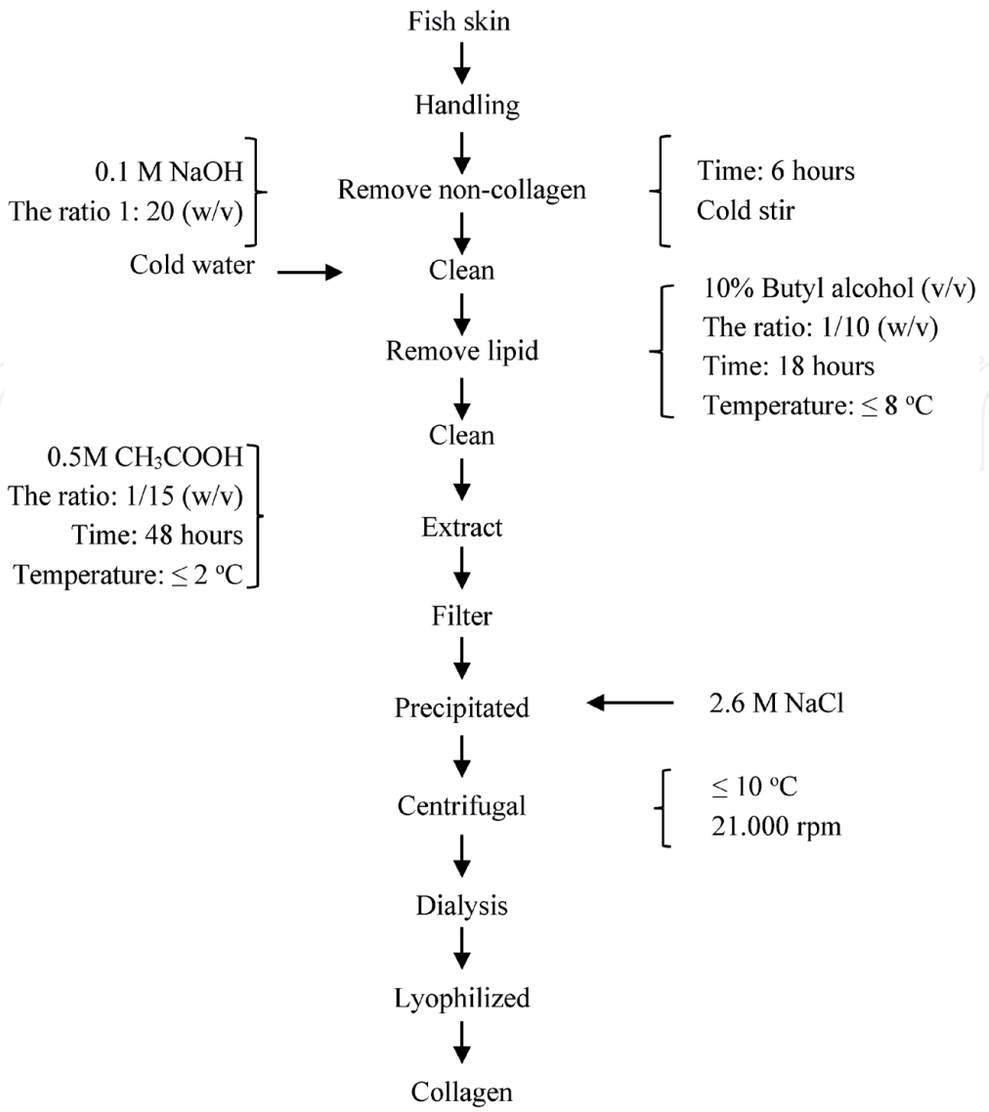

Figure 6.
Extraction schematic of antioxidant collagen from fish skin.

is 4.19% (calculated on the wet weight of the skin), the obtained collagen is types I that consisted of 2 chains α is α_1 and α_2, denaturation temperature is 31.16°C. According to the study, pangasius skin was treated with 0.2 M NaOH for 66 hours, extracted collagen in acetic acid 0.37 M for 2.5 days. Collagen precipitation occurs in 2.05 M NaCl in 4 minutes. The results showed collagen collection efficiency of 31.16%.

4.2 Polysaccharide

All over methods could use for polysaccharides extraction, but only three solvents could use, for example, alkaline, acid, and aqueous.

4.2.1 Fucoidans

For fucoidans extraction, removing the pigment and the lipid out of brown algae is by using an organic solvent such as ethanol/acetone/chloroform and HCl, respectively. Before the fucoidans extraction, separation of polyuronic acid is also via the other steps, such as neutralize (8% $NaHCO_3$), concentration, dialysis (10 kDa membrane), and precipitation. Finally, fucoidans are collected with HCl solvent and running the DEAE cellulose (**Figure 7**).

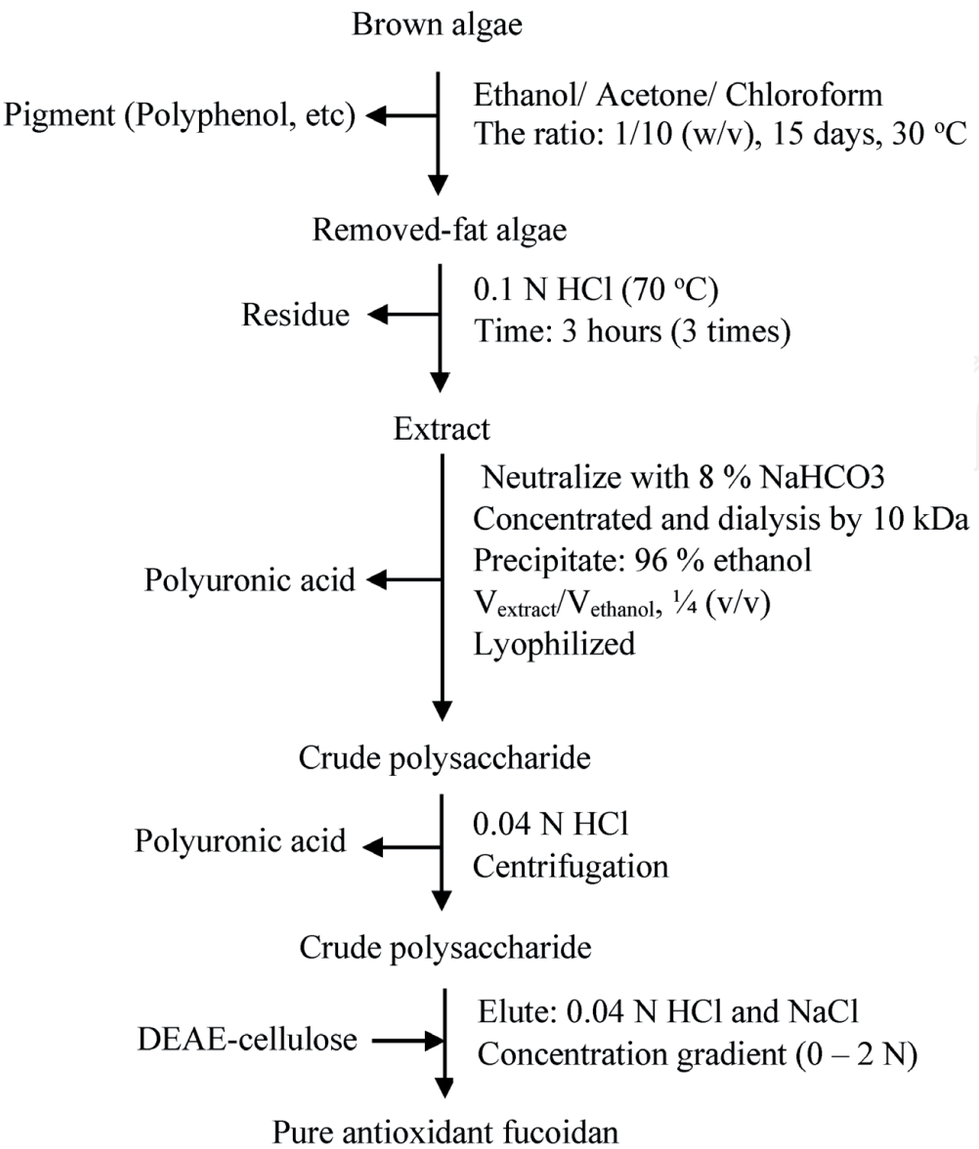

Figure 7.
Extraction schematic of antioxidant fucoidan from brown algae.

4.2.2 Alginates

The removal step of pigment and lipid by using an organic solvent and HCl could not be lack. Na_2CO_3 is a useful solvent for the extraction of alginate. The conversion of sodium alginate to calcium alginate is necessary. Calcium alginate still links to phlorotannins, so bleaching is important. Acidification (pH 2) is phlorotannins removal. Finally, the conversion of sodium alginate is from alginic acid, and the precipitation of them by using 80% ethanol (**Figure 8**) [70].

Based on experiments, the use of a hydrochloric solvent with flexibility in terms of temperature, the material-to-solvent ratio, and time, as well as neutralization, dialysis, concentration, precipitation, centrifugal, and drying, will allow obtaining small units of alginate such as sodium mannuronate and sodium guluronate (**Figure 9**).

4.2.3 Glucosamine

Shrimp shells contain many chitin, protein, and minerals, so removing protein and minerals using HCl and NaOH are indispensable steps. After removing proteins and minerals, continue to wash and soak chitin in HCl solvent to hydrolyze

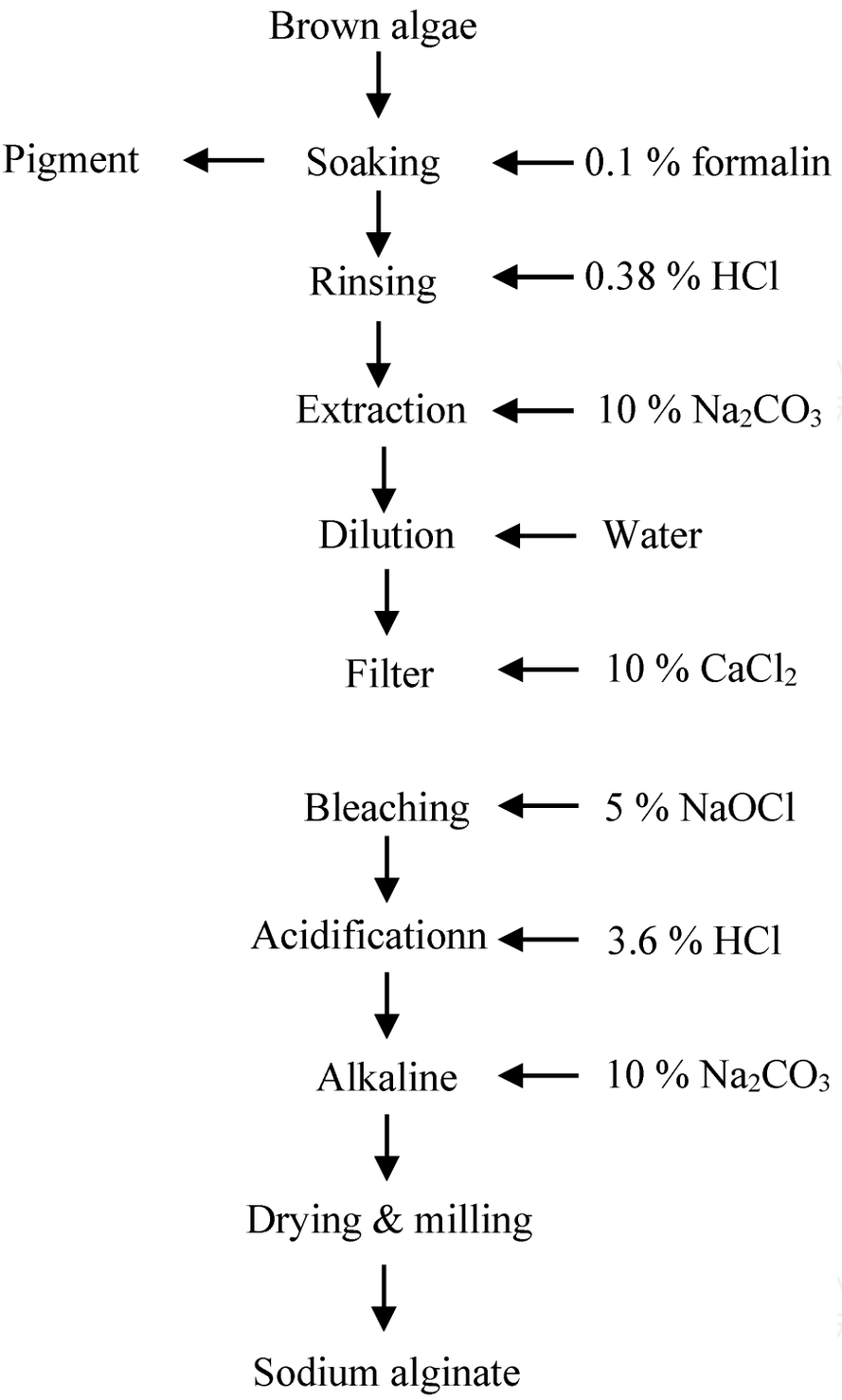

Figure 8.
Extraction schematic of antioxidant alginate from brown algae.

glucosamine. Glucosamine hydrochloride will be collected by crystallization with ethanol and dried at 50°C (**Figure 10**) [71].

4.2.4 Inulins

Extracting and purifying inulin from dangshen, color separation is also a necessary step from the beginning. After color separation, inulin is extracted with water and undergoes filtration, concentration, and purification using ethanol, activated carbon, and $CaCl_2$. The above repetitions will be useful for purified inulin absorption (**Figure 11**).

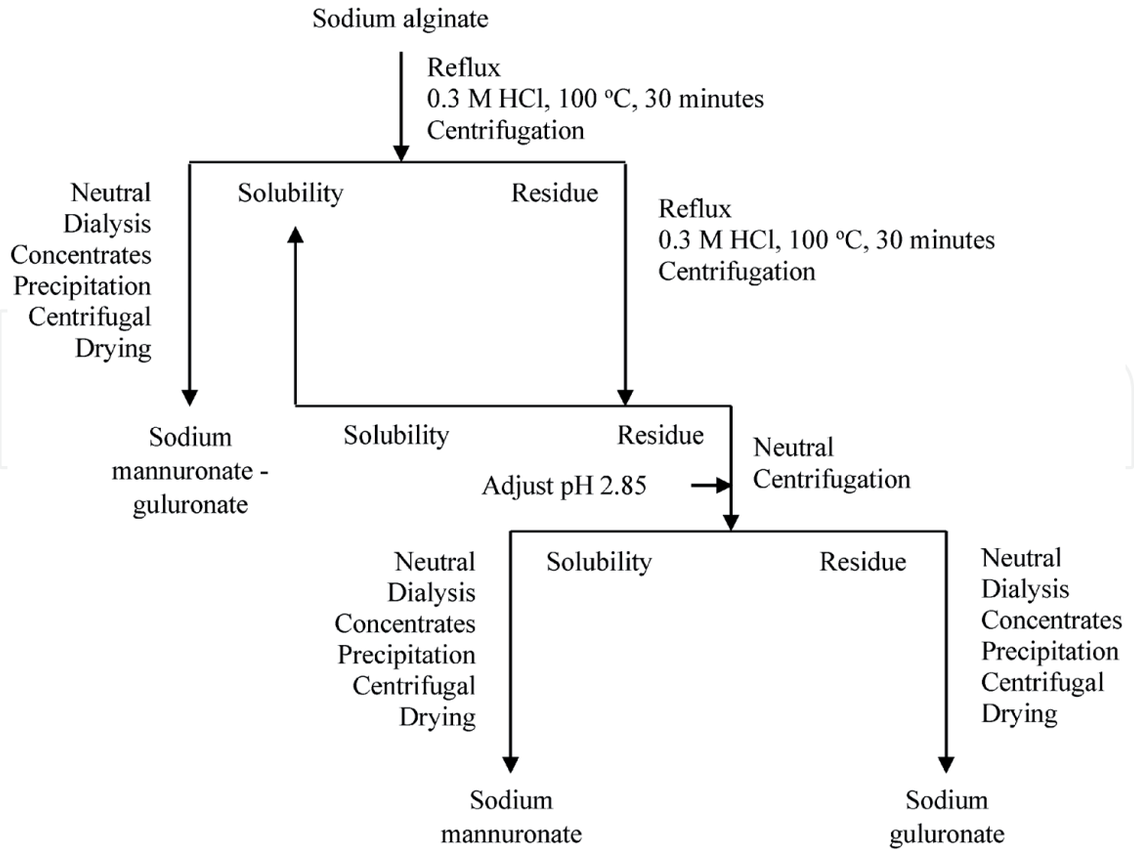

Figure 9.
Extraction schematic of antioxidant sodium mannuronate and sodium guluronate from alginate.

4.2.5 Laminarins

As laminarin is a water-soluble polysaccharide sulfate, several proteins, fucoidan, and alginate also exist in the laminarin extract. Protein removal is using trichloroacetic acid. Eliminate alginate and fucoidan are by adjusting pH and mass fractionation, respectively (**Figure 12**) [72].

4.2.6 Ulvans

Use dichloromethane and ethanol for the most removal of the lipids and pigment that exist in Ulva. Hot-water extractions of the pre-treatment algae are for 7 hours at 75–85°C under continuous stirring. The filtration and centrifugation are for collecting the supernatant. Removal of starch and proteins is continuously by using enzymatic hydrolysis. Afterward, running the solution is via activated charcoal for centrifuging, filtering, and precipitating with absolute ethanol. Finally, the precipitate is ulvan (**Figure 13**) [73].

4.2.7 Pectins

The first step in plant pectin extraction is always the color removal step with organic solvents. After de-colorant from the plants, pectin extraction is by using the solvents with different pH. The filtrate is concentrated and hydrolyzed to remove protein and starch. At this time, semi-pectin was collected and continued to run through activated carbon to remove impurities. Finally, 80% of ethanol is useful for precipitation and purification of pectin (**Figure 14**).

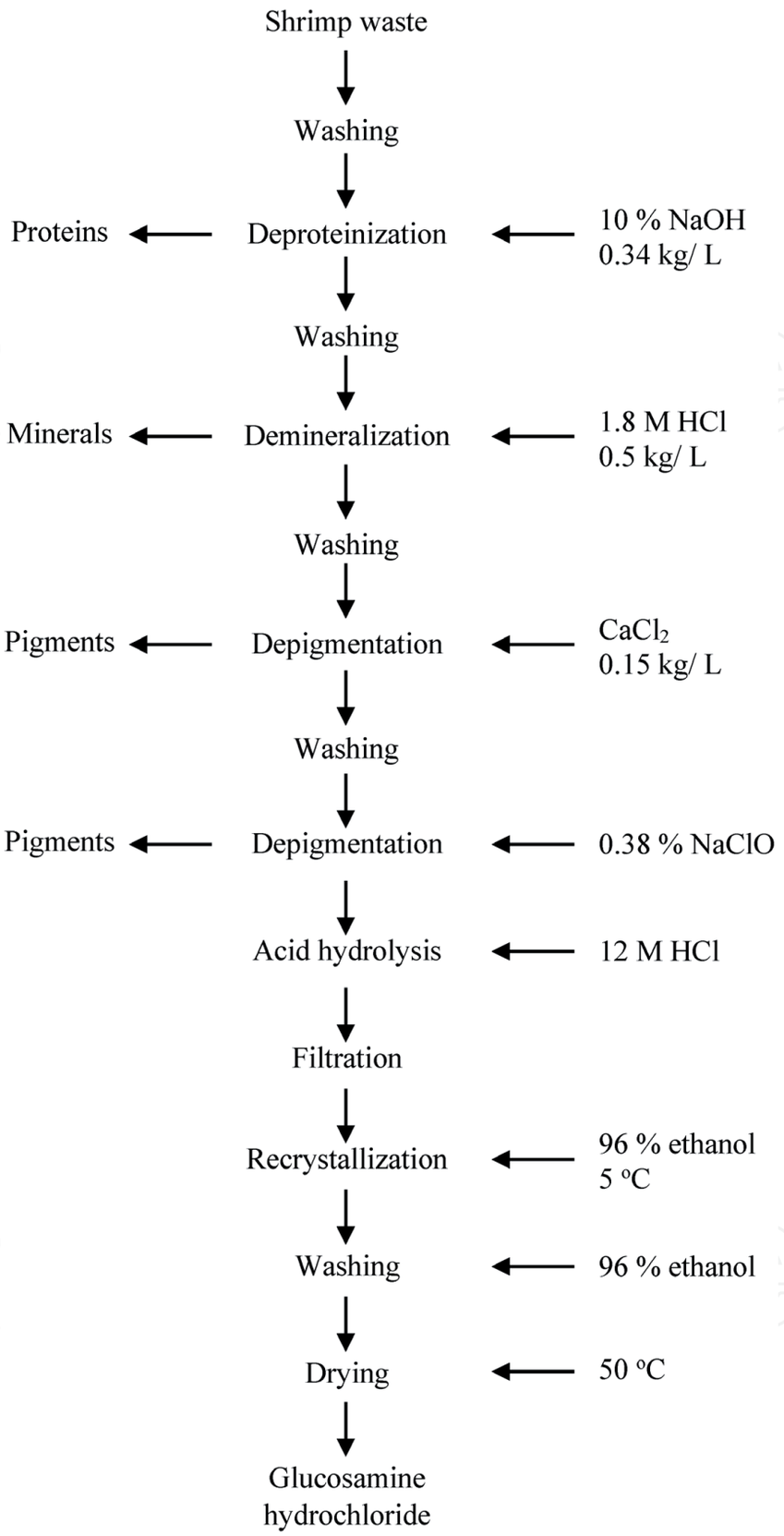

Figure 10.
Extraction schematic of antioxidant glucosamine hydrochloride.

4.3 Secondary metabolites

4.3.1 Polyphenols

Phlorotanins dissolve into the organic solvent, such as ethanol, methanol, ethyl acetate. Phlorotannins in brown algae grown in Vietnam mainly dissolve in ethanol

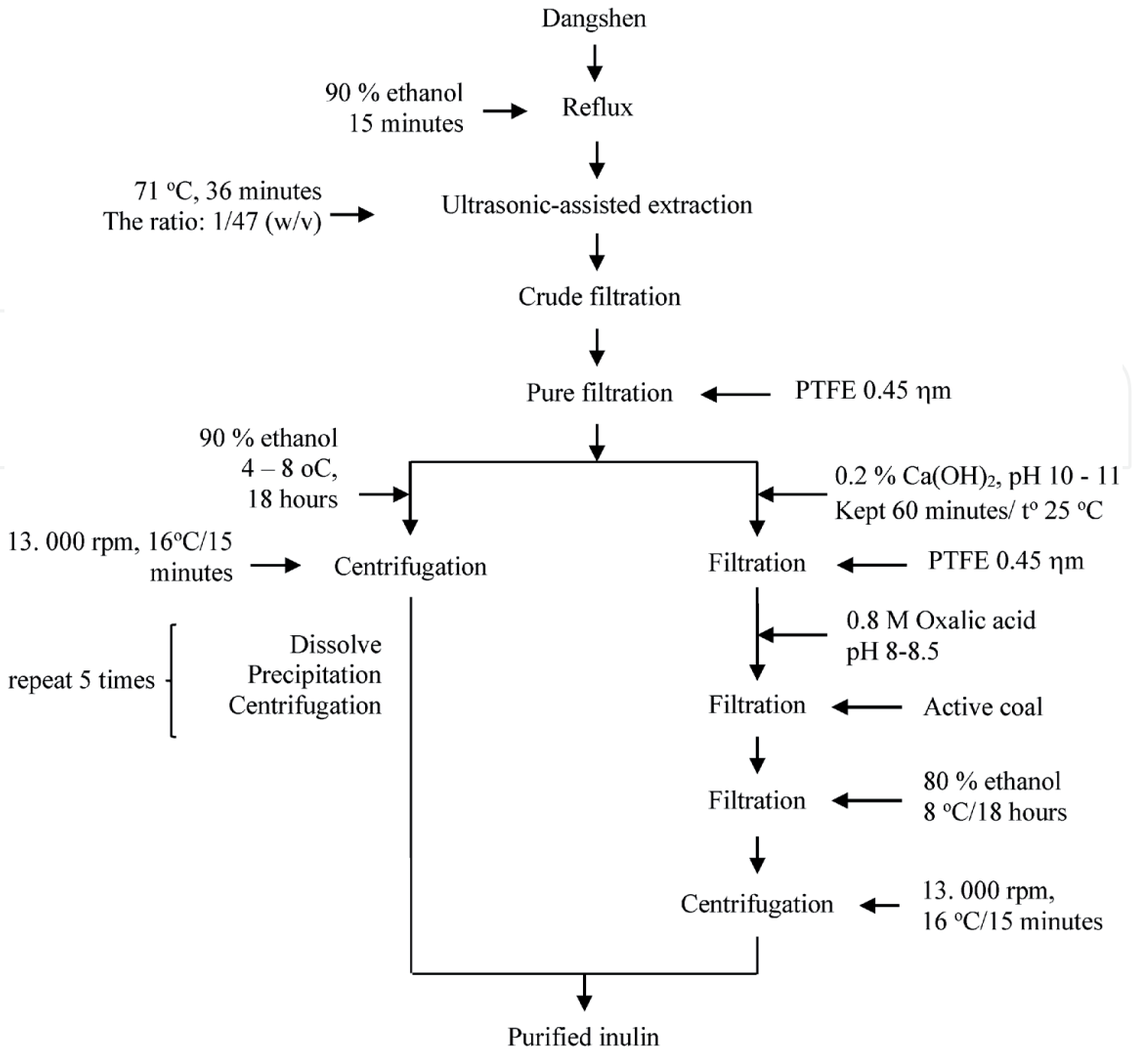

Figure 11.
Extraction schematic of antioxidant inulin from dangshen.

and focus on the fraction ethyl acetate. Sephadex LH20 is useful for the purification of phlorotannins (**Figure 15**). For polyphenol of terrestrial plants also used ethanol as a useful solvent.

According to Neeraj et al., lignins exist in various four styles, such as kraft, lignosulfonate, organosolv, and soda [74, 75].

There are three methods for the extraction of lignin; (i) 7.5% NaOH, (ii) organosolv (85% formic acid/85% acetic acid), and (iii) poly-ethylene glycol (PEG). Method (ii and iii) use heating assistance.

In method (i), the material-to-solvent ratio of 1/10 (w/v) at 90 ± 2°C for 90 min with pH (12) of the black liquor is useful for the extraction of lignin. After hot filtration and allowing to cool for the precipitation is using acidification with 0.5 M H_2SO_4. Meanwhile, the current authors extracted successful kraft lignins from corn stalks according to the **Figure 16**. This difference can be from material differences.

In method (ii), the condition is as follows: Formosolv/acetosolv ratio of 70/30 (v/v), the biomass-to-solvent ratio of 1/8 (w/v) for 2 hours at 98 ± 2°C and allowing to cool for filtration. The residue cleaning is with 80% formic acid and distilled water. The dilution of black liquor is with distilled water, stirred, centrifuged for 1 hour.

In method (iii): Lignin extraction is with the material-to-solvent (1% (w/w) 98% H_2SO_4) ratio of 1/4 (w/v) at 160 ± 2°C for 2 hours and down to room temperature for the collection of the supernatant. The residue washing is with 1,4-dioxane and removed 1,4-dioxane in the black liquor by rotary evaporation.

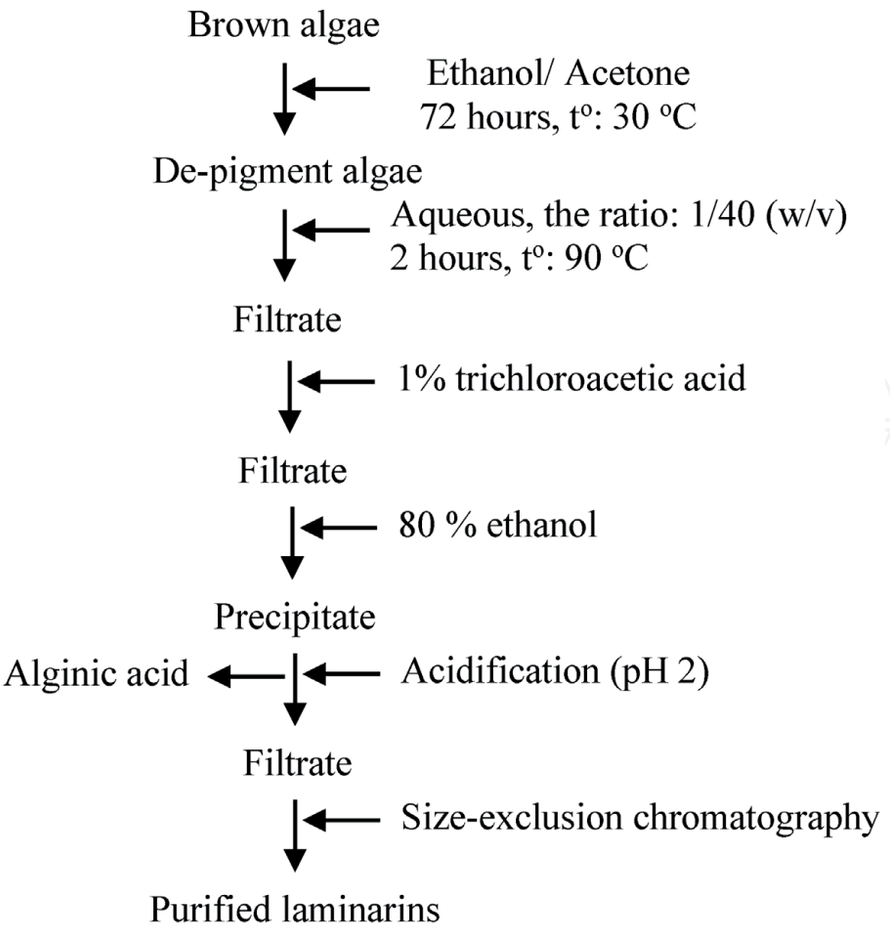

Figure 12.
Extraction schematic of antioxidant laminarin from brown algae.

During the black liquor dilution by using distilled water, stir and centrifuge are for 1 hour. The precipitated lignin(s) is separated, neutral with distilled water, and oven-dried at 50°C for 48 hours.

4.3.2 Alkaloids

Alkaloids are one of the main photochemical components of plants, so they are also extracted with organic solvents, and most claims indicate that alkaloids are soluble in ethanol. According to Surya et al., alkaloid extract after chasing ethanol solvent added 5% CH_3CO_2H, filter, and separated with CH_2Cl_2. The aqueous phase is collected and adjusted to pH 10 for the continuous fraction with CH_2Cl_2. Finally, the obtain of CH_2Cl_2 fraction because alkaloids exist in the CH_2Cl_2 phase (**Figure 17**) [76].

4.3.3 Flavonoids

About 5000 flavonoids have been identified and noticed. Each group of substances in flavonoids can dissolve in different solvents. Apigenin-7-methyl ether and flavone aglycone are among the substances found in 70% ethanol extracts that support the boiling point of water after the plant pre-treatment with Petroleum ether at 40 to 60°C. Ethyl acetate fractionated with ethyl acetate to obtain the ethyl acetate fraction. Then run via the polyamide column, which will select the purified Apigenin-7-methyl ether and flavone aglycone (**Figure 18**) [77].

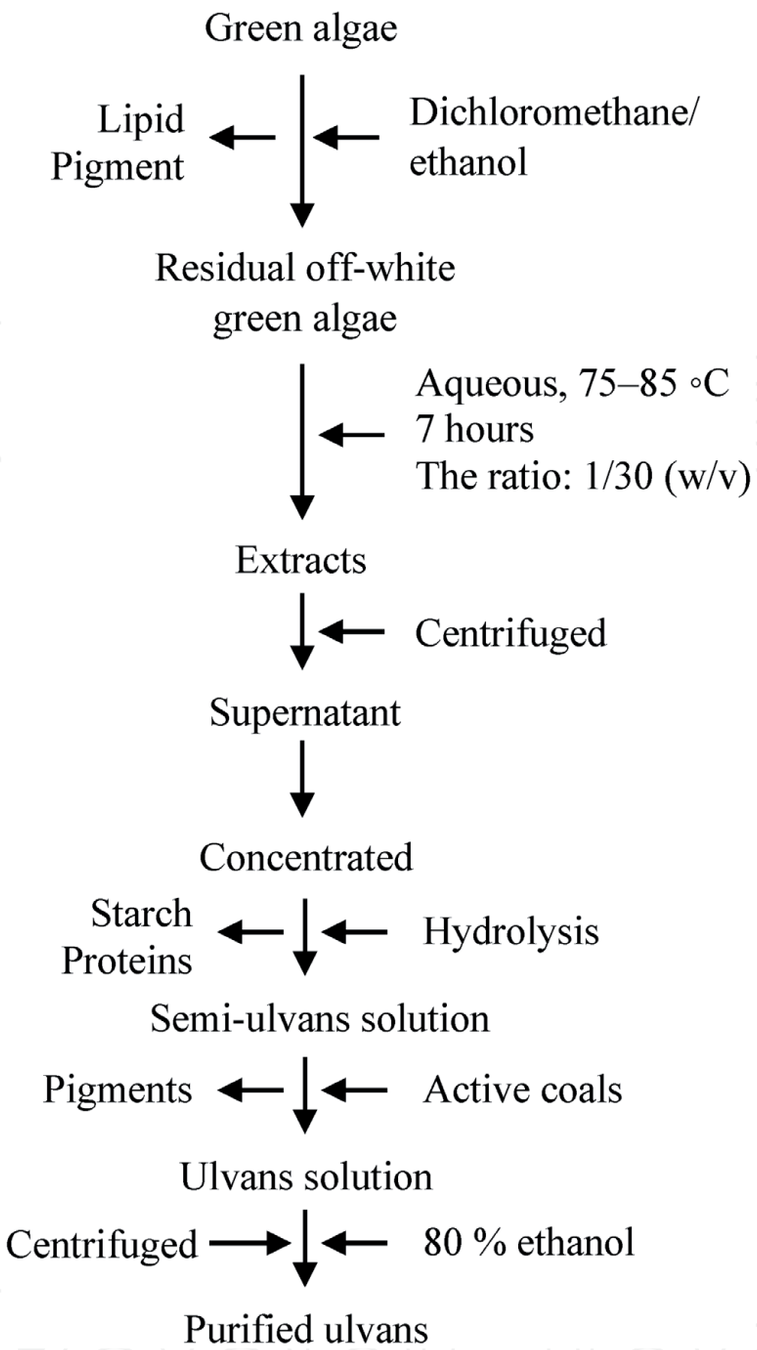

Figure 13.
Extraction scheme of antioxidant ulvan from green algae.

5. Production technology of functional-antioxidant food

5.1 The capsules

In capsules, the preparation of an emulsion system is necessary for filling into the capsules. The emulsion contains antioxidants, surfactants, and excipients that increase the antioxidant effects of functional food. The capsule shells are always composed of gelatin, sorbitol, and colorant ingredients (**Figure 19**).

Some excipients are common in the preparation of the capsule as follows (**Table 1**):

The typical emulsion viscosity is from 50 to 1000 Centipoise (cP). The melting temperature does not exceed 70°C. The suitable particle size in the emulsion is

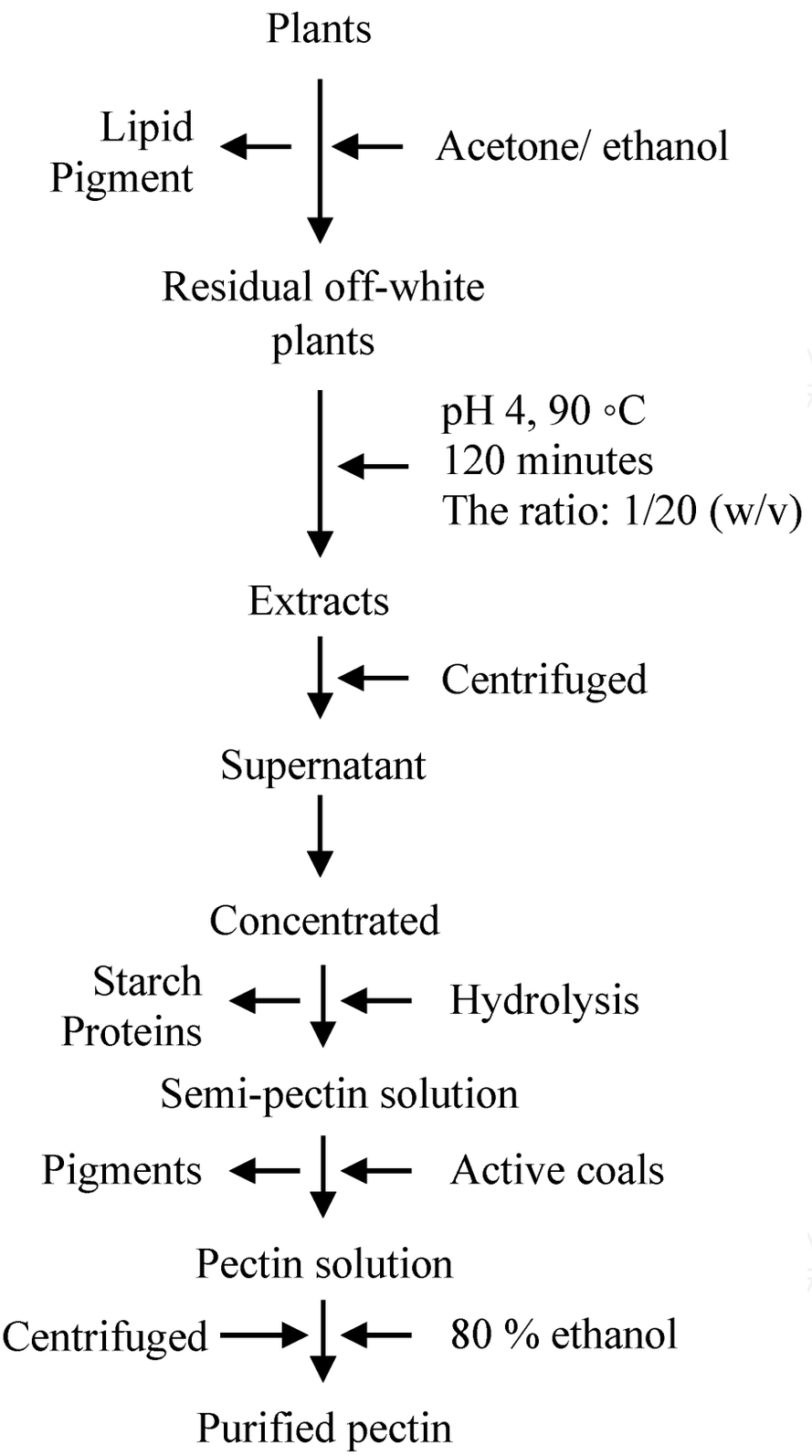

Figure 14.
Extraction scheme of antioxidant pectin from plants (Cactus).

less than 20 μm. The formula gets the standard when Phenomena such as stringing, dripping, splashes, or solidification does not happen at the dosing nozzle. The emulsion should be solidifying below 40 °C. For two-piece capsules, the compaction force is typically useful of 20–30 N, compared to tableting (10–30 kN) (**Figure 20**).

The manufacture of hard gelatin capsules by using a dip-coating method involves four stages. (i) Dipping solution (the gelatin solution preparation), (ii)

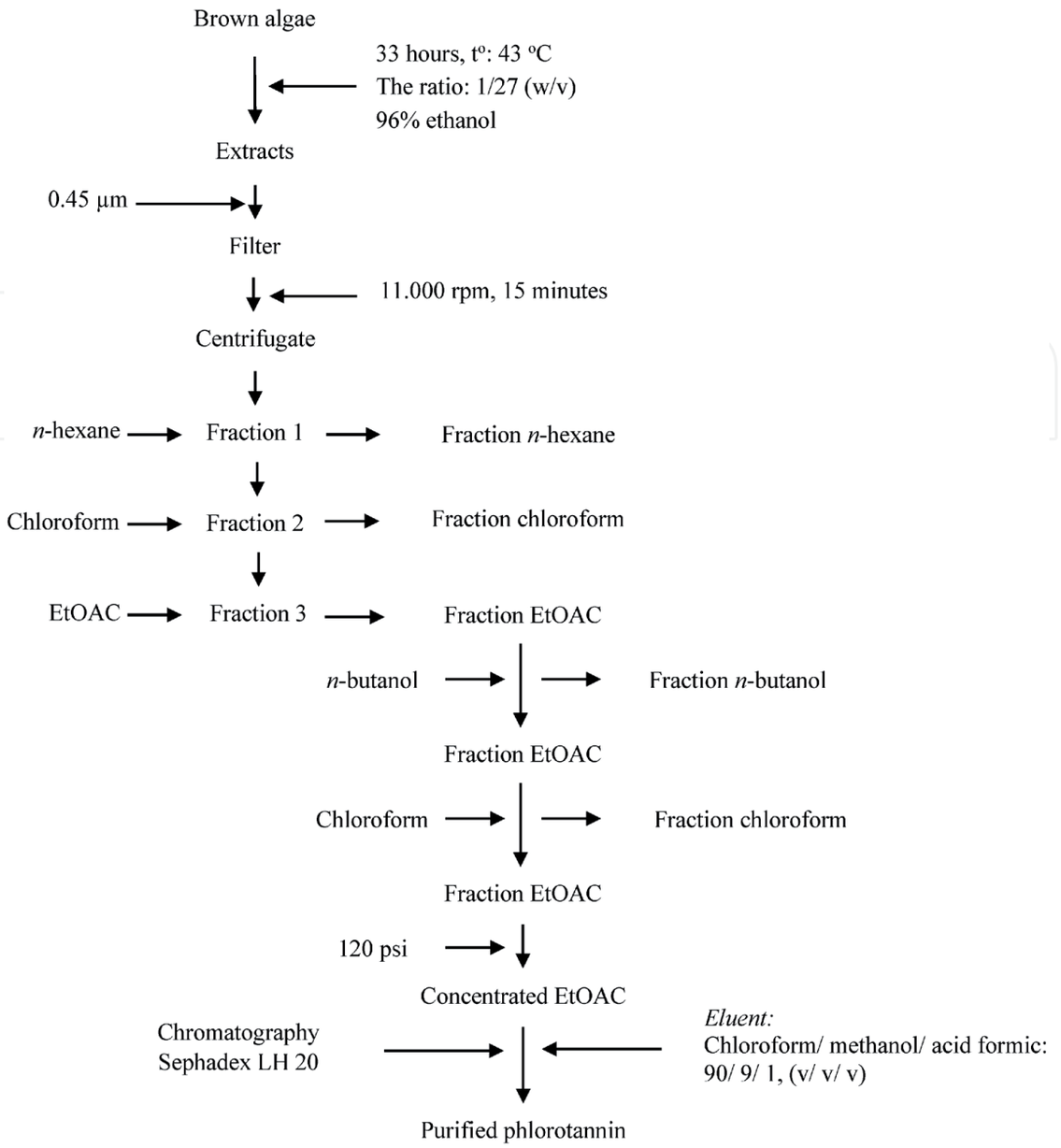

Figure 15.
Extraction scheme of antioxidant phlorotannins from brown algae.

Gelatin-coating on metal pins, rotation, and pins drying, (iii) Stripping, trimming, and the capsule shell joining; (iv) Printing [81].

5.2 The tablets

Functional-antioxidant food tablets contain various powder components that ensure the characteristics of consistency, flow, cohesion, and porosity) for the guarantee of the size, half-life, and swelling capacity of the tablets. All tablets have to get uniforms in the tablet weight, antioxidant content, the indication requirements, and storage time [81].

The actual tablet weights (175 mg) need the target force of 9500 N [82] (**Figure 21**).

5.3 The tubes

For the tube, the compositions of functional-antioxidant food is required in the liquid or the serum and respond all indexes according to the standards.

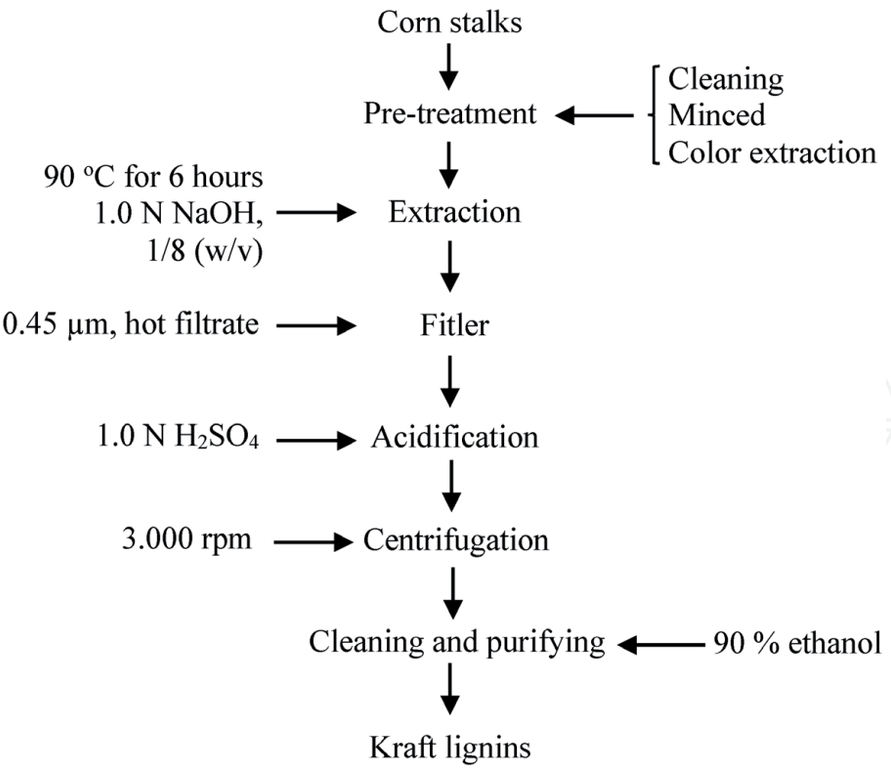

Figure 16.
Extraction scheme of antioxidant lignins from corn stalks.

5.3.1 The effervescent tablets

The effervescent tablet has different compositions such as tartaric acid citric acid, sodium bicarbonate, potassium citrate, mannitol, sorbitol, aspartame, talc. For some formulations, in the granulation process, polyvinylpyrrolidone plays a role as a binder. The wet granulation is suitable for the production of effervescent tablets composed of potassium citrate [83]. The compression and uniformity of effervescent tablets in the wet granulation technique is better and gets less error in the processing such as sticking, capping, and friction than other methods. The strawberry-raspberry flavor is useful for effervescent tablets. All effervescent tablets must contain bicarbonate to make CO_2 [84].

For effervescent tablets of phloroglucinol (dihydrate), the formulation is as follows: phloroglucinol dihydrate (80.0 mg), citric acid (297.2 mg), sodium bicarbonate (362.6 mg), and sodium benzoate (15.2 mg) [85].

5.3.2 The powders and the hard pills

Figures 22 and **23** exhibit the production process for the powder and hard pills of antioxidant polyphenol, chlorophyll from by-product maizes, respectively. The current process is similar, compared to the tablets and the capsules, but their shapes are various. Hard pills are popular in Vietnam.

6. Mechanism of functional-antioxidant food

The antioxidant activity of hydrolyzed collagen depends mainly on the presence of hydrophobic groups in the peptide chains. Histidine and aromatic amino acids play a controlling role in antioxidant activity via two mechanisms for the de-activation of free radicals: (i) hydrogen atom transfer (hydrogen donation in the

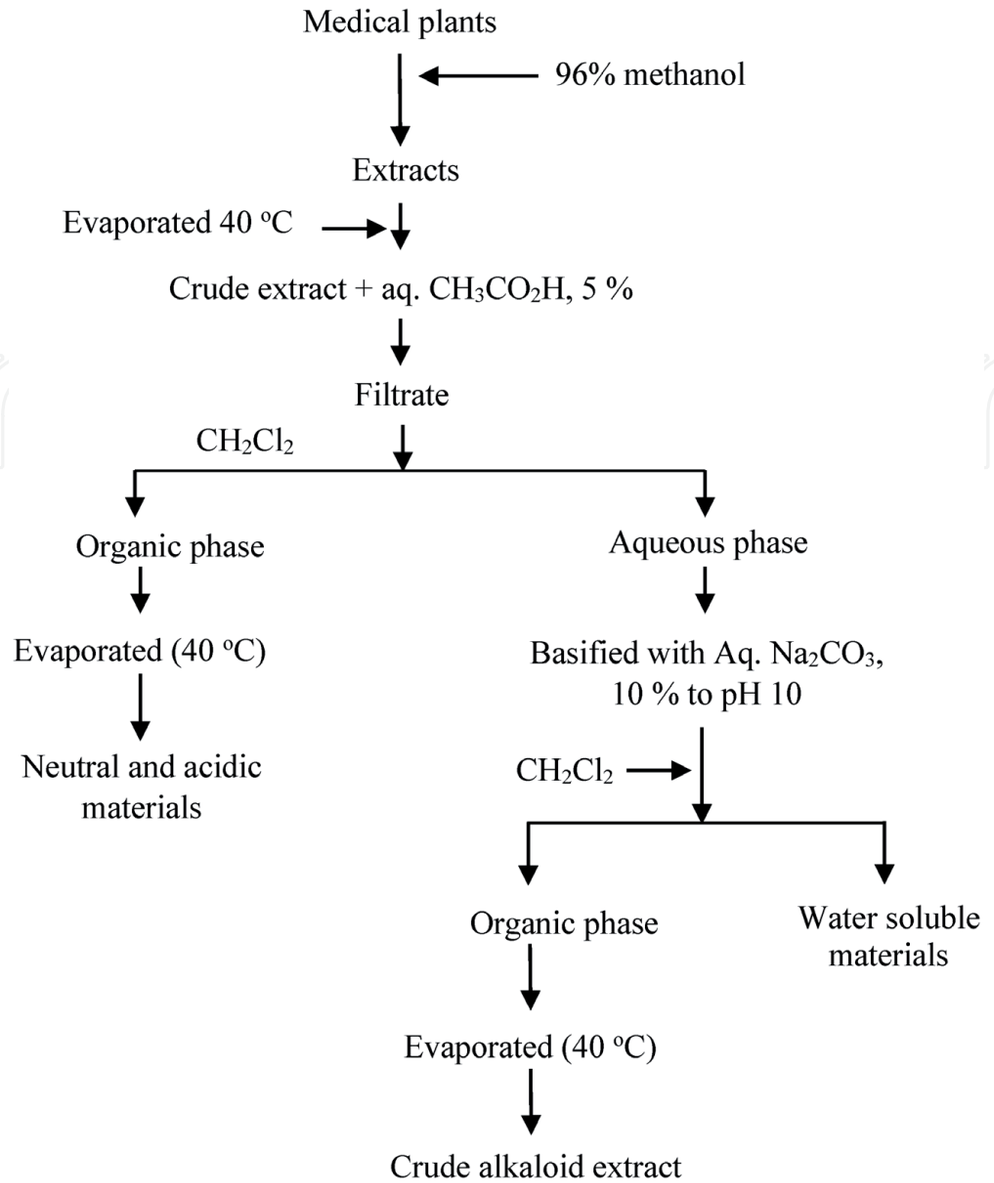

Figure 17.
Extraction scheme of antioxidant alkaloid extract from plants.

assays of ORAC and TRAP), (ii) single electron transfer (one-electron transfer to reducing agent in the assays of DMPD and FRAP assays). The antioxidant capacity of tyrosine is basing on the mechanism (i) and pathway (ii) is mostly for the group (cysteine and histidine) [86, 87].

The mechanism of antioxidant activity is basing on the generation prevention of free radicals via the pathway of chelating ions (ferrous and copper). Transition metal ions take apart to reactive to superoxide and hydrogen peroxide in Fenton reaction for the reactive hydroxyl radicals formation ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^-$). The hydroxyl radical scavenging capacity and the chelating ability of polysaccharide depend on their structure. Some hypotheses on the antioxidant activity of polysaccharides estimate dissociation energy decrease of the hydrogen bond, the abstraction activation of the anomeric carbon, and reduction of molecular weight. For the sulfated polysaccharide, the sulfate groups lead to acidification and weaken the hydrogen bond between other polysaccharides [88, 89].

In general, antioxidant molecules usually take part in the redox reaction as a reducing agent. •NO generation happens under the impact of nitric oxide-synthase on intracellular arginine and combines to O_2 to the formation of $ONOO^•$ that cause lipid peroxidation. The peroxidation is also one of the reasons causing autoimmune

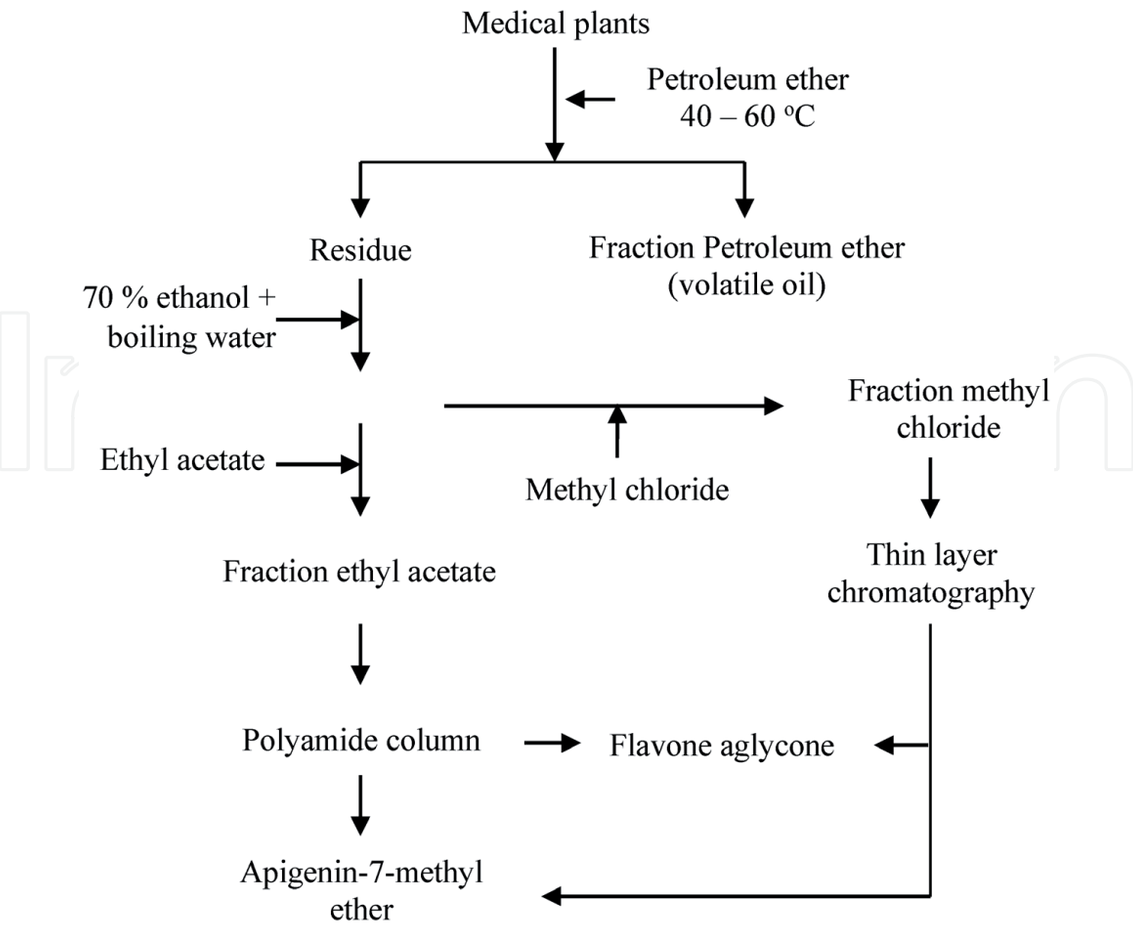

Figure 18.
Extraction scheme of antioxidant flavonoids from plants.

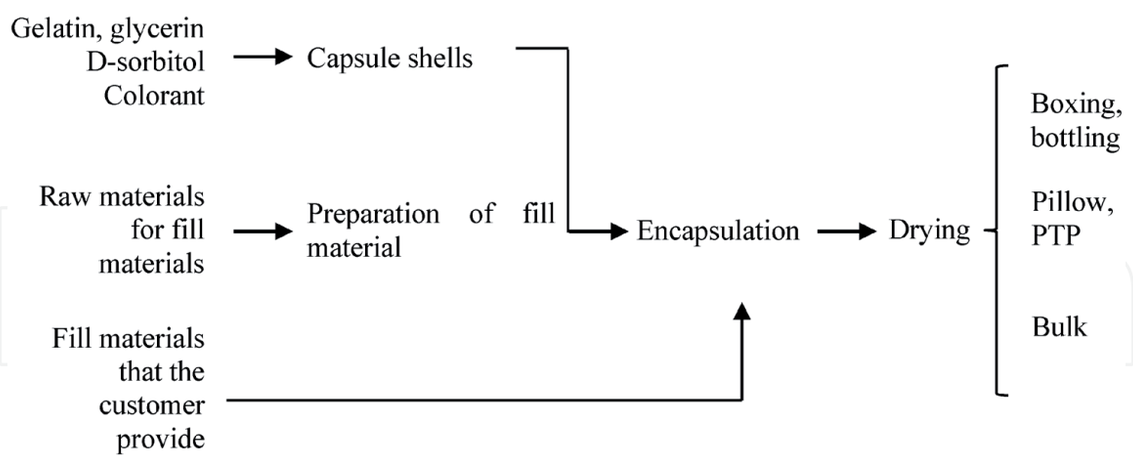

Figure 19.
The production shematic of functional-antioxidant capsules.

diseases (rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes, scleroderma, multiple sclerosis, psoriasis, and vitiligo). The metals deactivation and lipid hydroperoxides could be via the antioxidants increase, the singlet oxygen elimination, and the undesirable volatiles minimize. The peroxyl radical ($ROO^{\bullet}$) scavenging mechanism of polyphenol is basing on free radicals getting hydrogen cations of polyphenol and forming hydrogen bonds. The antioxidant activity decrease in a hydrogen-bond-rich medium [90].

Excipient	Emulsion	Substances
Lipophilicity	Vegetable oils	Corn oil, Castor oil, Sesame oil, Olive oil, Hydrogenated vegetable oil, Peanut oil, Soybean oil, Fractionated coconut oil
	Esters	Glycerol Stearate, Isopropyl myristate, Ethyl oleate, Glycol Stearate
	Fatty Acids	Laurie acid, Stearic acid, Oleic acid, Palmitic acid, Oleic acid
	Fatty Alcohols	Stearyl alcohol, Cetyl alcohol
Water-retaining		PEG 3000–6000 MW
Ambipolar		Lecithin, Poloxamers, PEG esters

Table 1.
Liquid excipients compatible with hard gelatin capsule shells [78, 79].

Figure 20.
Some gelatin capsules [80].

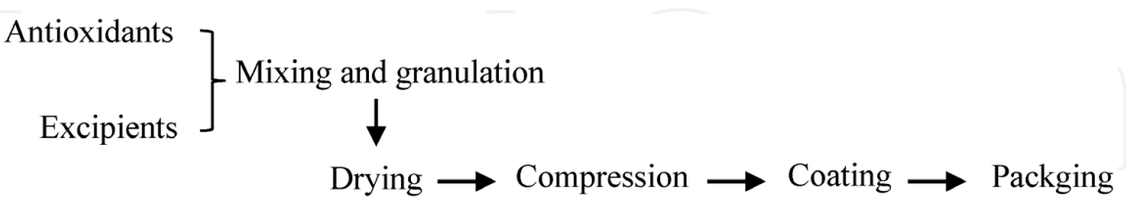

Figure 21.
The production shematic of functional-antioxidant tablets.

7. Opportunity and challenge

Nowadays, in the development trend of the world for surviving and developing in parallel with various functional food and pharmaceutical products in different countries, functional-antioxidant foods have to face numerous opportunities and challenges, such as the technology and science advancement, the aging increase, and expanding global trade. Aging is the biggest threat to humans and causes about 80 different diseases in the world. It is the growth of aging populations, environmental pollution, and life pressures that leads to disease and economic burdens. With

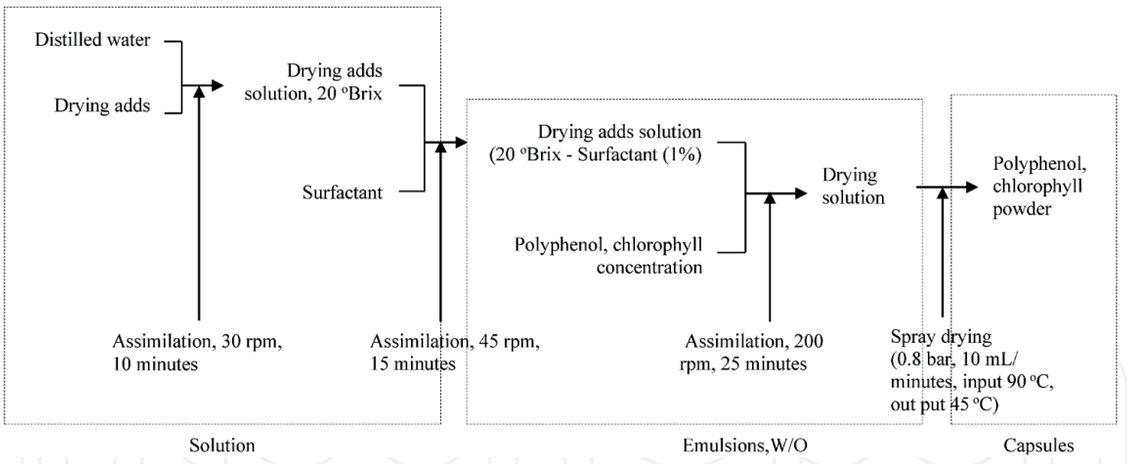

Figure 22.
The production shematic of functional-antioxidant powder from by-product maizes.

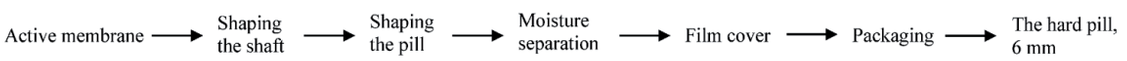

Figure 23.
The production shematic of functional-antioxidant hard pill from by-product maizes.

Beverages	Antioxidant content	Nuts, legumes and grain products	Antioxidant content	Berries, fruit and vegetable	Antioxidant content
Apple juice	0.27	Barley, pearl and flour	1.0	Bilberries, dried	48.3
Apple juice	0.92	Barley, pearl and flour	0.5	Amla (Indian gooseberry), dried	261.5
Orange juice	0.64	Buckwheat, whole meal flour	2.0	African baobab tree, leaves dry, crushed	48.1
Pomegranate juice	2.1	Chestnuts, with pellicle	4.7	Dog rose, products of dried hip	69.4
Prune juice	1.0	Maize, white flour	0.6	Zereshk, red sour berries	27.3
Red wine	2.5	Walnuts, with pellicle	21.9	Chilli, red and green	2.4
Tomato juice	0.48	Pecans, with pellicle	8.5	*Moringa Stenopetala*, dried leaves, stem	11.9

Table 2.
Statistical descriptives of antioxidant content in some food (mmol/100 g) [91].

the development of science and technology, antioxidant bioactive substances are exploited more effectively and thoroughly. However, the uniformity of science and technology in various regions does still not happen. Awareness increase, economy,

and global trade ability lead to the criteria of the goods choice of human more difficult. The difference in antioxidant content in various foods occurs. These are both opportunities and challenges to develop antioxidant supplement products (**Table 2**).

8. Conclusions

This chapter gave an overview of the antioxidant supplements based on the international knowledge and experimentation of the authors, from the basic understanding of the antioxidant active groups (8 groups of polysaccharides, 01 group of proteins, and 05 groups of secondary metabolites) and the origin of these to the technological process of extracting and producing antioxidants to their opportunities and challenges in today's world are all demonstrated.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Alugoju P, Dinesh BJ, Latha P. Free radicals: Properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 2015;30(1):11-26. DOI:10.1007/s12291-014-0446-0

[2] Charles D Ciccone. Free-radical toxicity and antioxidant medications in Parkinson's disease. Phys Ther. 1998; 78(3):313-319. https://doi.org/10.1093/ptj/78.3.313

[3] Dahir RdAJ, Rodrigo BdS, Sânia AdS, Dahir RdA. Oxygen free radicals and pulmonary disease. J Bras Pneumol. 2005;31(1):60-68. https://doi.org/10.1590/S1806-37132005000100011

[4] Denham H. Free radical theory of ageing: Applications. The Asia Pacific Heart Journal. 1998;7(3):169-177. https://doi.org/10.1016/S1328-0163(98)90023-9

[5] Ekaterina G, Donika I, Zhivko Z, Rumiana B, Maya G, Ichio A. Mitochondrial dysfunction and redox imbalance as a diagnostic marker of "Free Radical Diseases". Anticancer. 2017;37(10):5373-5381. DOI: 10.21873/anticanres.11963

[6] Joe MM. The evolution of free radicals and oxidative stress. Physiology In Medicine. 2004;108(8):P652-659. https://doi.org/10.1016/S0002-9343(00)00412-5

[7] Lien AP-H, Hua H, Chuong P-H. Free radicals, antioxidants in disease and health. Int J Biomed Sci. 2008;4(2):89-96. PMC3614697

[8] Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010;4(8):118-126. DOI: 10.4103/0973-7847.70902

[9] Lagouge M, Larsson N-G. The role of mitochondrial DNA mutations and free radicals in disease and ageing. J Intern Med. 2013; 273(6):529-543. DOI: 10.1111/joim.12055

[10] Masoud A. Oxidative stress and free radicals in liver and kidney diseases. J Nephropathol. 2018;7(3):127-131. DOI: 10.15171/jnp.2018.30

[11] Narendra M. Diseases related to types of free radicals. IntechOpen; 2019. DOI: 10.5772/intechopen.82879

[12] Pawan KS, Neelam K, Vince P, Dinender K. The role of oxidative stress in the genesis of heart disease. Cardiovasc Res. 1998;40(3):426-432. https://doi.org/10.1016/S0008-6363(98)00244-2

[13] Silpak B, Rintu D, Ena RB. 2017; 4(4):596-614. Role of free radicals in human inflammatory diseases. AIMS Biophysics. DOI: 10.3934/biophy.2017.4.596

[14] Wulf D. Free radicals in the physiological control of cell function. Physiological Reviews. 2002;82(1):47-95. https://doi.org/10.1152/physrev.00018.2001

[15] Irwin AT. Free radical reactions in food. 1984;61(4): 313-324. J Chem Educ. https://pubs.acs.org/doi/pdf/10.1021/ed061p313

[16] David RV-R, Jaime L-C, Dalia ISM. Tilapia (*Oreochromis aureus*) collagen for medical biomaterials. In: Sabyasachi M, editors. Seaweed biomaterials. IntechOpen; 2018. DOI: 10.5772/intechopen.77051

[17] Tiago HS, Joana M-S, Ana LPM, Alberta D, Yves B, Rui LR. Marine origin collagens and its potential applications. Mar Drugs. 2014;12:5881-5901. DOI: 10.3390/md12125881

[18] Mala N, Hanifah HH, Euis K, Eni K, Yoshihiro O. Characterization and antioxidant activity of collagen, gelatin, and the derived peptides from yellowfin tuna (*Thunnus albacares*) skin. Mar Drugs. 2020;18(2):98-100. DOI: 10.3390/md18020098

[19] Jun KL, Sang IK, Yong JK, Min JK, Min SH, Byeong DC, Jin-Soo K. Comparison of collagen characteristics of sea- and freshwater-rainbow trout skin. Food Sci Biotechnol. 2016;25(1): 131-136. https://doi.org/10.1007/s10068-016-0020-z

[20] Khong NM, Yusoff FM, Jamilah B, Basri M, Maznah I, Chan KW, Armania N, Nishikawa J. Improved collagen extraction from jellyfish (*Acromitus hardenbergi*) with increased physical-induced solubilization processes. Food Chem. 2018;251:41-50. https://doi.org/10.1016/j.foodchem.2017.12.083

[21] Lei W, Thilina UJ, Hye-Won Y, Hyo GL, Min-Cheol K, Sanjeewa KKA, Jae YO, You-Jin J. Isolation, characterization, and antioxidant activity evaluation of a fucoidan from an enzymatic digest of the edible seaweed, *Hizikia fusiforme*. Antioxidants (Basel). 2020;9(5):363-377. DOI: 10.3390/antiox9050363

[22] Pham DT, Roza VM, Svetlana PE, Stanislav DA, Bui ML, Tatiana NZ. Structural characteristics and anticancer activity of fucoidan from the brown alga *Sargassum mcclurei*. Mar Drugs. 2013; 11(5):1456-1476. DOI: 10.3390/md11051456

[23] Micheline CRdS, Cybelle TM, Celina MGD, Fernando RFdS, Hugo AOR, Edda LL. Antioxidant activities of sulfated polysaccharides from brown and red seaweeds. J Appl Phycol. 2007;19(2):153-160. DOI: 10.1007/s10811-006-9121-z

[24] Yu W, Maochen X, Qi C, Aiguo J, Hao L, Shuliang S. Biological activities of fucoidan and the factors mediating its therapeutic effects: A review of recent studies. Mar Drugs. 2019;17:183-201. DOI: 10.3390/md17030183

[25] Annette B, Tina J, Dirk M, Mette MN, Thorsten JSB, Anne SM, Michael BR, Xiaoru H, Bodo S, Cordula G, Anne BB. Crude fucoidan content in two North Atlantic kelp species, *Saccharina latissima* and *Laminaria digitata*—seasonal variation and impact of environmental factors. J Appl Phycol. 2017;29(6):3121-3137. DOI: https://doi.org/10.1007/s10811-017-1204-5

[26] Stefan K. Algal polysaccharides, novel applications and outlook. In: Chuan-Fa Chang Carbohydrates, editors. Comprehensive studies on glycobiology and glycotechnology. IntechOpen; 2012. DOI: 10.5772/2702

[27] Hanne HT, Jan K. Alginate in drug delivery systems. Drug Dev Ind Pharm. 2002;28(6):621-630. https://doi.org/10.1081/DDC-120003853

[28] Kuen YL, David JM. Alginate: properties and biomedical applications. Prog Polym Sci. 2013;37(1):106-126. https://doi.org/10.1016/j.progpolymsci.2011.06.003

[29] Wenxiao J, Wenxue C, Yuqi M, Yonghuan Y, Boqiang W, Qiuping Z, Haiming C, Weijun C. Effects of molecular weight and guluronic acid/mannuronic acid ratio on the rheological behavior and stabilizing property of sodium alginate. Molecules. 2019;24:4374-4386. DOI: 10.3390/molecules24234374

[30] Sabrine S, Islem Y, Hanen BAd, Hana M, Veronique M, Marguerite R, Mostefa D, Tahar M, Mohamed H, Moncef N. Structural, physicochemical and antioxidant properties of sodium alginate isolated from a Tunisian brown seaweed. Int J Biol Macromol. 2015;72: 1358-1367. https://doi.org/10.1016/j.ijbiomac.2014.10.016

[31] Dang XC, Dang TTT, Do TK. Biophysical-chemistry characterization of alginate in brown algae species *Sargassum dupplicatum*. World Journal of Food Science and Technology. 2020;4(1):17-22. DOI: 10.11648/j.wjfst.20200401.13

[32] Jörg J. Effects of glucosamine and chondroitin sulfate on cartilage

metabolism in OA: Outlook on other nutrient partners especially omega-3 fatty acids. Int J Rheumatol. 2011;Article ID 969012:17 pages. DOI:10.1155 /2011/969012.

[33] Xing R, Liu S, Wang L, Cai S, Yu H, Feng J, Li P. The preparation and antioxidant activity of glucosamine sulfate. Chin J Oceanol Limnol. 2009;27(2):283-287. DOI: https://doi.org/10.1007/s00343-009-9135-x

[34] Dalirfardouei R, Karimi G, Jamialahmadi K. Molecular mechanisms and biomedical applications of glucosamine as a potential multi-functional therapeutic agent. Life Sci. 2016;152:21-29. DOI: 10.1016/j. lfs.2016.03.028

[35] Tathiana SMM, Ângelo SMM, Daniel ERF, Gisela MDO. Bio-technological production of oligo-saccharides — Applications in the food industry. In: Ayman HAE, editors. Food production and industry. IntechOpen; 2015. DOI: 10.5772/60934

[36] Valentina P, Annamaria A\, Michele PLG, Vittoria L, Silvia C, Sara C, Rossella P, Rossana A, Laura DG, Michele C. Antioxidant activity of inulin and its role in the prevention of human colonic muscle cell impairment induced by lipopolysaccharide mucosal exposure. PLoS One. 2014;9(5):e98031. DOI: 10.1371/journal.pone.0098031

[37] Laurie-Eve R, Sylvie T, Martin B. Structural characterization of laminaran and galactofucan extracted from the brown seaweed *Saccharina longicruris*. Phytochemistry. 2010;71(13):1586-1595. DOI: 10.1016/j.phytochem.2010.05.021

[38] Spicer SE, Adams JMM, Thomas DS, Gallagher JA, Winters AL. Novel rapid method for the characterisation of polymeric sugars from macroalgae. J Appl Phycol. 2017;29(3):1507-1513. DOI: 10.1007/s10811-016-0995-0

[39] Gaurav R, Rajeev R, Marco G-V, Dilip KR, Torres S, John O'D. Molecular characteristics and antioxidant activity of laminarin extracted from the seaweed species *Laminaria hyperborea*, using hydrothermal-assisted extraction and a multi-step purification procedure. Food Hydrocoll. 2021;112:106332-106342. https://doi.org/10.1016/j.foodhyd. 2020.106332

[40] Dang XC, Laminarin (Beta-glucan) of brown algae *Sargassum mcclurei*: extraction, antioxidant activity, lipoxygenase inhibition activity, and physicochemistry properties. World Journal of Food Science and Technology. 2020;4(1):31-39. DOI: 10.11648/j.wjfst. 20200401.15.

[41] Joel TK, Marie M, Rocky dN, Christopher RKG. Ulvan: A systematic review of extraction, composition and function. Algal Res. 2019;39:101422. https://doi.org/10.1016/j.algal.2019. 101422

[42] Huimin Q, Tingting Z, Quanbin Z, Zhien L, Zengqin Z, Ronge X. Antioxidant activity of different molecular weight sulfated poly-saccharides from *Ulva pertusa* Kjellm (Chlorophyta). J Appl Phycol. 2005; 17(6):527-534. https://doi.org/10.1007/ s10811-005-9003-9

[43] Nasrul W, Chu YS, Wong YS, Tina R, Raden BI, Rimadani P, Muchtaridi M. Characterization and antioxidant activity of pectin from Indonesian mangosteen (*Garcinia mangostana* L.) rind. 2019; 5(8):e02299. https://doi.org/10.1016/j.heliyon.2019.e02299

[44] Vasily VS, Victoria VG, Fedor VV, Olga AP, Nikolay YS, Olga GS, Sergey VP. The antioxidant properties of pectin fractions isolated from vegetables using a simulated gastric fluid. 2017;Article ID 5898594. https://doi.org/10.1155/2017/5898594

[45] Khasina EI, Kolenchenko EA, Sgrebneva MN, Kovalev VV,

Khotimchenko YS. Antioxidant activities of a low etherified pectin from the seagrass *Zostera marina*. Russ J Mar Biol. 2003;29:259-261. https://doi.org/10.1023/A:1025493128327

[46] Yajing L, Xiaoting F, Delin D, Xiaoyong L, Jiachao X, Xin G. Extraction and identification of phlorotannins from the brown alga, *Sargassum fusiforme* (Harvey) Setchell. Mar Drugs. 2017;15(2):49-64. DOI: 10.3390/md15020049

[47] Dang XC, Vu NB, Tran TTV, Le NH. Effect of storage time on phlorotannin content and antioxidant activity of six Sargassum species from Nhatrang Bay, Vietnam. J Appl Phycol. 2016;28:567-572. DOI: https://doi.org/10.1007/s10811-015-0600-y

[48] Vu NB, Dang XC, Phan TKV. Effects of extraction conditions over the phlorotannin content and antioxidant activity of extract from brown algae *Sargassum serratum* (Nguyen Huu Dai 2004). Free Radicals and Antioxidants. 2016;7(1):115-122. DOI: 10.5530/fra.2017.1.17

[49] Jayachandran V, Kishor KK, Sukumaran A, Ira B, Se-Kwon K. Phlorotannins. encyclopedia of food chemistry. 2019;515-527. DOI: 10.1016/B978-0-08-100596-5.22360-3

[50] Riitta K, Jyrki L, Tuija H, Veijo J. Contents of soluble, cell-wall-bound and exuded phlorotannins in the brown alga *Fucus vesiculosus*, with implications on their ecological functions. J Chem Ecol. 2005;31(1):195-212. https://doi.org/10.1007/s10886-005-0984-2

[51] Marcelo DC, Artur MSS, Susana MC. Fucaceae: A source of bioactive phlorotannins. Int J Mol Sci. 2017;18(6):1327-1357. DOI: 10.3390/ijms18061327

[52] Herminia D. Functional ingredients from algae for foods and nutraceuticals. In: Herminia D, editor. Food science, technology and nutrition. Woodhead; 2013. p. 768.

[53] Sista KAK, Qin W. Structural and functional properties of pectin and lignin–carbohydrate complexes de-esterases: a review. Bioresour Bioprocess. 2018;5(43):1-16. DOI: https://doi.org/10.1186/s40643-018-0230-8

[54] Javier UH-B, Inty OH-DL, Mónica MC-S, Alexia S-L, Fernando H-T, Nagamani B. Insight into pretreatment methods of lignocellulosic biomass to increase biogas yield: Current state, challenges, and opportunities. Appl Sci. 2019;9(3721):1-29. DOI: https://doi.org/10.3390/app9183721

[55] Abla A, Basma EK-H, Dominik B, Michael L, Birgit K, Steffen W, Margit S. Lignocellulosic biomass as source for lignin-based environmentally benign antioxidants. Molecules. 2018;23(10):2664-2669. DOI: 10.3390/molecules23102664

[56] Peter XC, Gale GB, José AF-C, Massimo FM, Peter KP, Yao T, Bing Z, Ronghua L, Rong T. Free and conjugated phenolic compounds and their antioxidant activities in regular and non-darkening cranberry bean (*Phaseolus vulgaris* L.) seed coats. J Funct Foods. 2015;18(Part B):1047-1056. https://doi.org/10.1016/j.jff.2014.10.032

[57] Delile AMM, Carla M. Review ecological function of phenolic compounds from mediterranean fucoid algae and seagrasses: An overview on the genus *Cystoseira sensu lato* and *Posidonia oceanica* (L.). J. Mar Sci Eng. 2020;8:1-19. https://doi.org/10.3390/jmse8010019

[58] Jin D, Russell JM. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010;15(10):7313-7352. DOI: 10.3390/molecules15107313

[59] Deepak MK, Surendra SK, Mahabaleshwar VH, Hanhong B.

Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci. 2015;11(8): 982-991. DOI: 10.7150/ijbs.12096

[60] Jin G, Ying F, Zhao H, Xian L, Hong Z. Correlations between antioxidant activity and alkaloids and phenols of maca (*Lepidium meyenii*). J Food Qual, 2017;Article ID 3185945:10 pages. DOI: https://doi.org/10.1155/2017/3185945;

[61] İlhami G, Riad E, Akçahan G, Aun C, Fevzi T. Antioxidant activity of bisbenzylisoquinoline alkaloids from *Stephania rotunda*: Cepharanthine and fangchinoline. J Enzyme Inhib Med Chem. 2010;25(1):44-53. DOI: https://doi.org/10.3109/14756360902932792

[62] Lucia R, Magdaléna M, Daniela K, Milan S. Antiradical and antioxidant activities of alkaloids isolated from *Mahonia aquifolium*. Structural aspects. Bioorg Med Chem. 2004;12(17):4709-4715. DOI: 10.1016/j.bmc.2004.06.035

[63] Tian-yang W, Qing L, Kai-shun B. Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. Asian J Pharm Sci. 2018;13:12-23. https://doi.org/10.1016/j.ajps.2017.08.004

[64] Weisheng F, Zhiyou H, Meng L. Isolation and structure identification of flavonoids, flavonoids - from biosynthesis to human health. In: Goncalo CJ, editors. IntechOpen; 2017. DOI: 10.5772/67810

[65] Cong TN, Tuyen CK, Hoang TH, Duong HQ, Tien TN, Dinh HD, Dang XC. Phytochemistry, nutrient, mineral, and antioxidant activities of two species Morinda L. grown in three provinces in Vietnam. Sapporo Med J. 2020;54(05):1-8.

[66] Sulaiman CT, Balachandran I. Total phenolics and total flavonoids in selected indian medicinal plants. Indian J Pharm Sci. 2012;74(3):258-260. DOI: 10.4103/0250-474X.106069

[67] Daniela R. Flavonoids and the structure-antioxidant activity relationship. J Pharmacogn Nat Prod. 2018;4(1):1-2. DOI: 10.4172/2472-0992.1000e109

[68] Tian-yang W, Qing L, Kai-shun B. Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. Asian J Pharm Sci. 2018;13:12-23. https://doi.org/10.1016/j.ajps.2017.08.004

[69] Dwivedi S, Malik C, Chhokar V. Molecular structure, biological functions, and metabolic regulation of flavonoids. In: Gahlawat S, Salar R, Siwach P, Duhan J, Kumar S, Kaur P, editors. Plant Biotechnology: Recent advancements and developments. Springer; 2017. https://doi.org/10.1007/978-981-10-4732-9_9.

[70] Konda MNVSN, Singh S, Simmons BA, Klein-Marcuschame D. An investigation on the economic feasibility of macroalgae as a potential feedstock for biorefineries. Bioenerg Res. 2015;8:1046-1056. https://doi.org/10.1007/s12155-015-9594-1

[71] Martha B, Selene A, Luis M, Joaquín M. Production of glucosamine hydrochloride from crustacean shell. J Pharm Pharmacol. 2015;3:20-26. DOI: 10.17265/2328-2150/2015.01.003

[72] Heng Z, Kyung HR. Extraction and separation of polysaccharides from laminaria japonica by size-exclusion chromatography. J Chromatogr Sci. 2015;53:498-502. https://doi.org/10.1093/chromsci/bmu073

[73] Carina C, Anabela A, Paula RP, Rui AS, Eduardo ABdS, Rui LR, Alírio ER. Characterization of ulvan extracts to assess the effect of different steps in the extraction procedure. Carbohydr Polym. 2012;88:537-546. https://doi.org/10.1016/j.carbpol.2011.12.041

[74] Neeraj M, Aurélie C, François R, Stéphane G, Fabien S, Giulio M, Jin PG.

An overview on the use of lignin and its derivatives in fire retardant polymer systems. In: Matheus P, editor. Lignin - Trends and applications. InTech Open; 2018. DOI: 10.5772/intechopen.72963]

[75] Panamgama LA, Peramune PRUSK. Extraction and modification of lignin biopolymer. In: Moratuwa engineering research conference (MERCon); 29-31 May 2017; Sri Lanka. New York: IEEE; 2017. p. 13-18

[76] Surya H, John BB. Initial studies on alkaloids from lombok medicinal plants. Molecules. 2001;6(2):117-129. DOI: 10.3390/60100117

[77] Abd E-RAM, Hamdy AE-A, Mohamed AO, Sawsan AMN, Ismail MAN, Zakaria AT. Two flavonoid compounds isolated from *Nepeta septemcrenata* growing in south Sinai, Egypt. AJEthno. 2015;2(3):143-157.

[78] Robert GS. Solubilizing excipients in oral and injectable formulations. Pharm Res. 2004;21(2):201-231. https://doi.org/10.1023/B:PHAM.0000016235.32639.23

[79] Cristian V. Nutraceutical vegetable oil nanoformulations for prevention and management of diseases. Nanomaterials. 2020;10(6):1232-1264. DOI: https://doi.org/10.3390/nano10061232

[80] http://www.pharmaceutical-equipment.com/softgelmfg.htm

[81] https://www.pharmapproach.com/manufacture-of-pharmaceutical-tablets/

[82] Leo M, Jon H, Pablo V, Tim K. Tablet compression force as a process analytical technology (pat): 100 % inspection and control of tablet weight uniformity. J Pharm Sci. 2019;108(1):485-493. DOI: 10.1016/j.xphs.2018.07.004

[83] https://www.saintytec.com/tablet-manufacturing-guide/

[84] Abolfazl A, Fatemeh F. Formulation, characterization and physicochemical evaluation of potassium citrate effervescent tablets. Adv Pharm Bull. 2013;3(1):217-225. DOI: 10.5681/apb.2013.036

[85] https://patents.google.com/patent/DE60004204T2/en

[86] Gabriel A-C, Arely L-L, Verónica C-G, Rubén J-A, Gabriel A-Á. Collagen hydrolysates for skin protection: Oral administration and topical formulation. Antioxidants. 2020;9(2):181-198. DOI: 10.3390/antiox9020181

[87] Julia M, Eugenia MK, María JN, Agustín GA. Antioxidant capacity of anthocyanin pigments. In: Gonçalo J, editor. Flavonoids. 2007; DOI: 10.5772/67718

[88] Junqiao W, Shuzhen H, Shaoping N, Qiang Y, Mingyong X. Reviews on mechanisms of in vitro antioxidant activity of polysaccharides. Oxid Med Cell Longev. 2016;Article ID 5692852:13 pages. http://dx.doi.org/10.1155/2016/5692852

[89] Nathan RP, Julia LB. A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. Cell Biochem Biophys. 2009;53(2):75-100. DOI: 10.1007/s12013-009-9043-x

[90] Norma FS-S, Raúl S-C, Claudia V-C, Beatriz H-C. Antioxidant compounds and their antioxidant mechanism. In: Emad S, editor. IntechOpen; 2019. DOI: 10.5772/intechopen.85270

[91] Monica HC, Bente LH, Kari H, Siv KB, Steinar D, Laura S, Carol W, Haruki S, Yuko U, Chiho S, Ingrid B, Nega B, Walter CW, Katherine MP, David RJJr, Rune B. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. Nutrition Journal. 2010;9(3):1-11. DOI: 10.1186/1475-2891-9-3