Chapter

Extraction, Characterization, and Application of Agricultural and Food Processing By-Products

Saroat Rawdkuen and Sunantha Ketnawa

Abstract

By-products originating from agricultural and food processing are considerable disposal problem for the industry because these waste streams emerge in huge quantities and often have direct impact on the economy and environmental pollution. However, at the same time, it constitute a rich but yet underutilized source of valuable components, which may find application as ingredients in the food and non-food industries. As a result, numerous projects are currently directed toward the utilization of agricultural and food processing by-products such as animal-based (skin, bone, flesh, and internal organs) and plant-based biomaterials (fruit peels and seeds, rice bran, and etc.). In the present work, we would like to focus on the potentialities and the current research of the compounds and extracts deriving from agro-industrial disposable wastes in the food-related utilization. The potential of selected by-products as a source of bioactive/functional compounds in terms of emerging and conventional techniques for extraction, characterization, biological activity monitoring and application of the extracts or isolated compounds as functional food ingredients or bio-based materials for food packaging are highlighted. Considering environmental effect and economic loss, agricultural and food processing by-products should be utilized in various innovative processes in the cause of beneficial product derivation.

Keywords: by-products, fish industry, film, food packaging, fruit and vegetables, gelatin, legume seeds, oilseed, rice, utilization

1. Introduction

Thailand is situated in the heart of the Southeast Asian mainland, fertile, tropical country with monsoonal climate that reinforce agriculture both in land and off land [1]. Besides, Thailand owes high agricultural output to it alluvial soils and topography, plentiful water supplies, which allows several plantings a year. Agriculture has long been the mainstay of the Thai economy, with abundant natural resources, and the majority of the Thai population engages in agricultural practices. Agricultural products in Thailand can be classified into four types: (1) *crops*, such as rice (number one export item of the country), tapioca, natural rubber, and pineapple; (2) *forestry and timber products* (40% of the area in Thailand has been designated as forests); (3) *fishery products*, including freshwater and saltwater fisheries; and

(4) *livestock products* such as chicken, eggs, and pork, which are also in high demand in the domestic market and exported [2, 3].

Thailand is one of the world's leading exporters of rice and also a major exporter of shrimp. The major trading partners with Thailand in agricultural products, ranked by export value, are as follows: (1) rice (Nigeria, China, Japan, and the USA); (2) natural rubber (Benin, Malaysia, the United Kingdom, and Japan); (3) processed chicken (South Africa, Japan, the Netherlands, Canada, and Taiwan); (4) chilled or frozen shrimp (Cote d'Ivoire, the USA, Germany, and the Republic of Korea); and (5) tapioca products (Ghana, the Republic of Korea, Ireland, the United Kingdom, and Indonesia) [2]. Thailand's food processing industry has developed rapidly and is one of the most developed in Southeast Asia with more than 10,000 food and beverage processing factories. Thailand has a large fresh, frozen, and semi-cooked food industry. In 2017, food exports generated an income of more than US$26 billion or about 830 billion baht to the country, employing as many as 10.75 million workers in the agricultural and fishery sectors, which is expected to reach US$27.4 billion for 2019 [4, 5]. According to the Thai government, "Thailand ranks among the top of the world's food producing countries in several food categories". Thailand is therefore considered one of the world's important food exporting countries. The fast-growing demand for food by the world's population bodes well for the limitless expansion of the consumer market [2, 5, 6]. According to data supplied by the Trade and Economic Information Center, the Ministry of Commerce, the main groups of food export are as follows: (1) frozen seafood group, canned seafood, and processed seafood; (2) fresh chicken and products; (3) other food groups (including ready-to-eat food and semi-cooked food); (4) halal group or food and food products for Muslims, prepared as prescribed by Muslim law; (5) frozen vegetable and fruit group, canned vegetables and fruits, canned fruit in syrup, processed vegetables and fruits, and vegetable pickles [2].

In addition, Thailand government looks set to revitalize the Kitchen of the World campaign, a scheme to accelerate Thailand's plan to become a major world food exporter, as it aims to boost food exports to contribute >6% of the country's GDP. Initiated 15 years ago, the Kitchen of the World campaign aims not only to merely accelerate Thailand's plan to become a major world food exporter but also spearhead the export of products from the One Tambon One Product (OTOP) scheme. Thailand also approved the Strategic Framework for Food Management in Thailand (2017–2021), which aims to produce enough food to sustain domestic demand, support access to adequate food at all times, improve food quality, reduce food waste and use food correctly, promote sustainable food production, and support the development of food security and nutrition [7].

From the above information, number of production and exportation, and Thai government policy, we can understandably assume that a large amount of food materials as by-products, which are generated along the chain of food production and transformation, are thrown into the environment as consumption demand is growing. Not only Thailand concern, but the global food losses, by-products and wastes are also hot issue that needs to be taken into consideration. The highest percentage of by-products is found in fruits and vegetables, plus roots and tubers (45%), followed by fish and seafood (35%), and oilseed, meat, and dairy (20%), respectively [8]. According to Ezejiofor et al. [9], by-products from food manufacturing make up to 30–60% of the product that is utilized for human consumption and animal feeding. Most of the by-products commonly contain proteins, carbohydrates, and lipids, which are promising sources of value-added substances that can be extracted and utilized as a starting material for enzyme, gelatin, collagen, bioactive compounds, for example, enzymes and antioxidants extractions [10]. Current trends in the world as well as in Thailand are to recover and utilize the

food manufacturing by-products into useful materials as a means of achieving goal of sustainable development. Hence, considerable efforts in the valorization of agricultural and food processing residues have been made with the purpose of minimizing the amounts of by-products, reducing the environment pollution, and increasing sustainability of these by-products. This section reviews by-products from two main food processing industries such as animal-based biomaterials (fish skin, bone, flesh, and internal organs) and plant-based biomaterials (pineapple, mango, longan, tea, sacha inchi, oilseeds, legumes, rice, etc.) that have a potential to be produced as gelatin, seasoning powder, calcium powder, film, protein concentrate, isolate and hydrolysates, bioactive peptides, and others such as protease, tyrosinase, antioxidant, and antimicrobial agents.

2. Fish industry and by-products

Thailand is one of the top fish-producing nations in the world, supplying markets in the USA, Europe, and Asia. Fishery production in Thailand demonstrated a remarkable growth over the last three decades. The total production exceeded 0.7 (freshwater fish) and 3 million tons (marine fish) over the last 5 years. Thailand is now the largest exporter of canned tuna, chilled and frozen shrimp, shrimp products, chilled fish, and prawns. Accounting for 1.5% of various kinds of fishery products for exporting are fresh and frozen prawn, processed shrimp, squid, cuttlefish, fish fillet, and surimi and fish in the form of fresh, chilled-frozen, and processed food [11].

Freshwater fish are widely cultured for commercial exploitation and trade in Thailand. The Mekong giant catfish (*Pangasianodon gigas*) is one of the economically important farmed fish that has been successfully raised in Northern Thailand, especially in Chiang Rai province [11]. Recently, farmed giant catfish (GC) became an economically important cultured freshwater fish due to their quality attributes (i.e., texture, taste, color, and nutritive values). Morphology of Mekong giant catfish can be seen in **Figure 1**, and most farm-raised giant catfish are sold raw to restaurants, and in the near future, fish farmers hope to export its meat to other countries, especially in Asia and Europe. It is reported that fish-processing by-products after filleting account for ~75% of the total fish weight [12]. The viscera in some species of fish account for approximately 5–10% of the entire weight of the fish; the viscera percentage tends to increase with the fish body weight [12]. Nile tilapia, *Oreochromis niloticus*, is popular in freshwater aquaculture. In the global market, the demand for tilapia (TP) in all forms is increasing rapidly. More by-products have been produced from the expansion of the tilapia processing industry [13].

2.1 Fish-processing by-products

An increase in fish consumption has resulted in an increase in fish-processing by-products, and these, in turn, may contribute significantly to environmental pollution if there is no further valorization process. With an increase in fish processing, a large amount of internal organs and by-product are also generated. During the filleting, 50–65% of the body is discarded which are environmental pollutants. More than 60% of these residues, including of the bone/frame (12–17%), viscera (4–12%), heads (18–60%), cut-offs (6%), skin (4–7%), and roe (2%) are considered as waste as shown in **Table 1** and **Figure 2**. The waste is used in the production of fish meal, and in a very few cases, it is collected for the production of animal feeds; more frequently it is simply discarded. The utilization of these

Figure 1.
Farm and morphology of Mekong giant catfish.

Component	Yield (%)		
	Giant catfish	**Striped catfish**	**Nile Tilapia**
Whole fish	15–17 (kg)	4.1–4.6 (kg)	0.8–1 kg
Fillet skinned	50–52	42–45	4.6–6
Skin	4–6	5–7	4.5–6
Viscera	4–5	10–12	5–8
Head	25–27	18–20	52–60
Bone	12–15	15–17	—
Scale	—	—	2–3
Yield (%)	50–52	48–59	64–77
By-products (%)	45–53	48–56	64–77

Source: Vannabun et al. [14].

Table 1.
Fish-processing by-products of freshwater fish including giant catfish, striped catfish, and Nile tilapia.

by-products could make more profit to the producer and ease environmental problems. Besides, they will be benefit for the producer to gain more profit from their materials instead of discard. These by-products contain a significant amount of protein that could be converted into useful substances and used for food formulation and bioactive ingredients.

In order to upgrade the by-products of the fish industry, various systematic studies were made to fulfill the best utilization of fish-processing by-products. Fish skin, in particular, is a rich source of collagen and gelatin, and these by-products still contain a significant amount of protein-rich material [15–18]. Using proteases from fish viscera in combined with commercial enzymes can be an alternative way to reduce the enzymes costs [12, 14, 19]. Alternatively, digestive enzymes from shrimp or fish viscera can be recovered and be successfully used as processing aids, that is, for the production of gelatin hydrolysate with antioxidant activities and/or

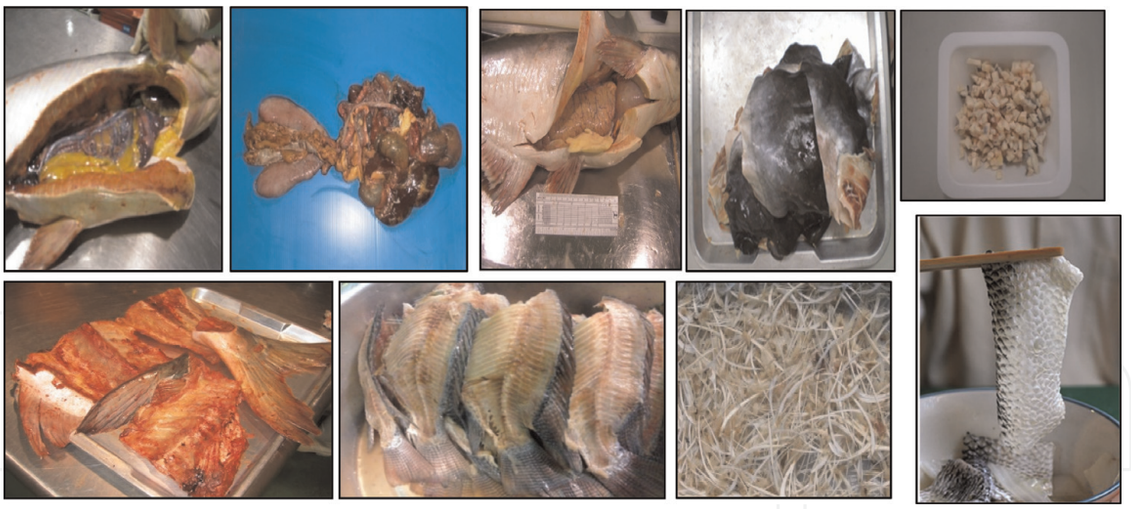


Figure 2.
Wastes and by-products of Nile tilapia and giant catfish. Source: Vannabun et al. [14].

angiotensin-I-converting enzyme (ACE) inhibitory activity [20, 21] or the extraction of carotenoprotein from shrimp waste [22]. Fish bone is another by-product that can serve as a raw material for the production of high value-added compounds that can be used in various sectors including agrochemical, biomedical, food, and pharmaceutical industries [23]. Fish bone is considered as one of the potential biological sources to produce calcium phosphates [23, 24]. Fish bone can be applied to boost nutritional characteristics in seasoning powder [25].

2.2 Utilization of fishery by-products

The growing consumer demand for healthy fish products has led to a thriving fish-processing industry worldwide. The fish-processing industry generates significant amounts of by-products and waste as illustrated in **Table 1** and **Figure 2** and tends to increase with fish body weight and consumption [26]. It is estimated that 25% of the global fish production is regarded as waste and is discarded or in the best case scenario processed into fish oil, fishmeal fertilizer, fish silage, and animal feed [27, 28]. It is costly to treat these wastes because it contains high content of organic matter. In addition, disposing these wastes may cause severe health and environmental issues as well as increase the cost of the fish industry.

2.3 Gelatin and collagen from fish skin

In general, gelatin is manufactured from the waste generated during animal slaughter and processing, that is, skin and bone. The most abundant gelatin sources are pig skin (46%), bovine hide (29.4%), and pork and cattle bones (23.1%) [29]. For several reasons, there are still serious concerns among the consumers to consume gelatin which is produced from bovine and porcine bones and skins. This is because of some problems such as religious matter and mad cow disease (bovine spongiform encephalopathy (BSE)) [16]. Thus, it lead to many researchers to discover alternative sources such as fish (marine and freshwater) [16, 18, 30] and poultry by-products [31]. As a consequence, there has been increasing interest in other sources, especially in fish skin and bone from fishery processing by-products. Gelatin was extracted from the skin of farmed giant catfish with a yield of 19.50 g/100 g skin sample on wet weight basis (**Figure 3**). The gelatin had high protein (89.1 g/100 g) but low fat (0.75 g/100 g) content and contained a high number of imino acids (proline and hydroxyproline) (211 residues per 1000

residues). Hydroxyproline accounted for 87 residues per 1000 residues. Giant catfish (GC) skin gelatin had a slightly different amino acid composition than calf (BF) skin gelatin. Gelatin was extracted from the skin of GC and tilapia (TP) at a yield of 19.50 and 23.34%, respectively. Gelatin from GC skin was lower in ash content (0.33 g/100 g). GC skin gelatin was rich in *nonpolar amino acid* glycine (35.9 g/100 g), alanine (10.6 g/100 g), and proline (12.4 g/100 g) [30].

Microstructures in transverse section photomicrographs of both GC and TP skin clearly projected the arrangement of collagenous bundles in each fish skin (**Figure 4**). TP showed a small bundle with the same ordered pattern, while in GC, the alternate arrangement was observed with a large bundle. The gaps between collagenous bundles represent the compact structure of the component in the skin. This particular characteristic affected the extractability of the collagen and the gelatin in the structure. The relative thickness of the two dermal layers depends on the presence or absence of scales, and it varies with the fish species. In general, the skin of animal is composed of collagen as a main compound. GC skin showed the large collagenous bundles with ordered arrangement when compared with the TP skin, while TP skin showed more compact and small bundles of collagenous components. These results cause different extraction yields of gelatin. The compact arrangement of the bundle effect to the strength and hardness of the fish skin [16].

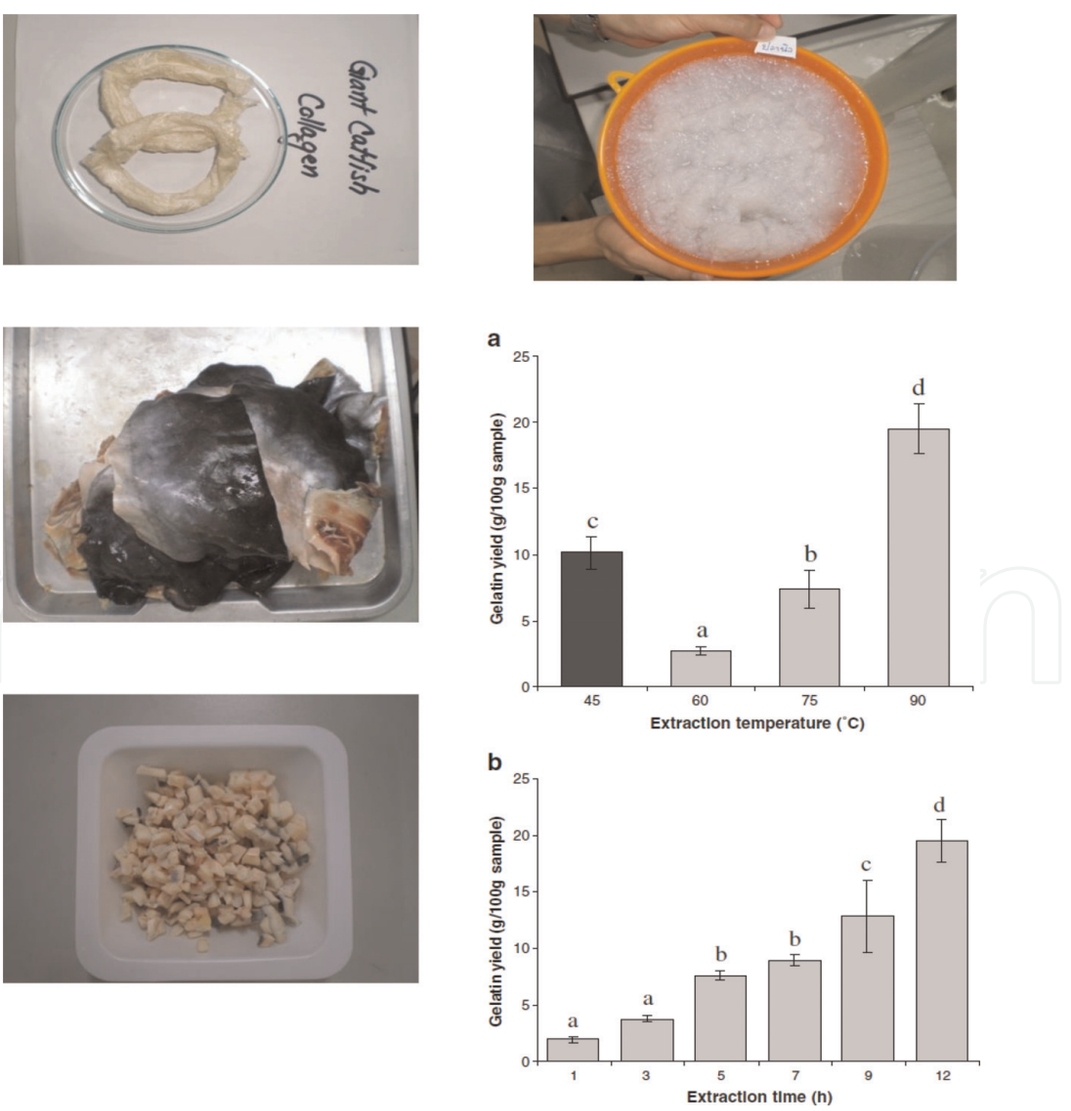


Figure 3.
Extraction of collagen and gelatin form farmed giant catfish skin. Sources: Rawdkuen et al. [16] and Sai-Ut et al. [18]. Different lowercase letters in the bar chart indicate significant difference ($p < 0.05$).

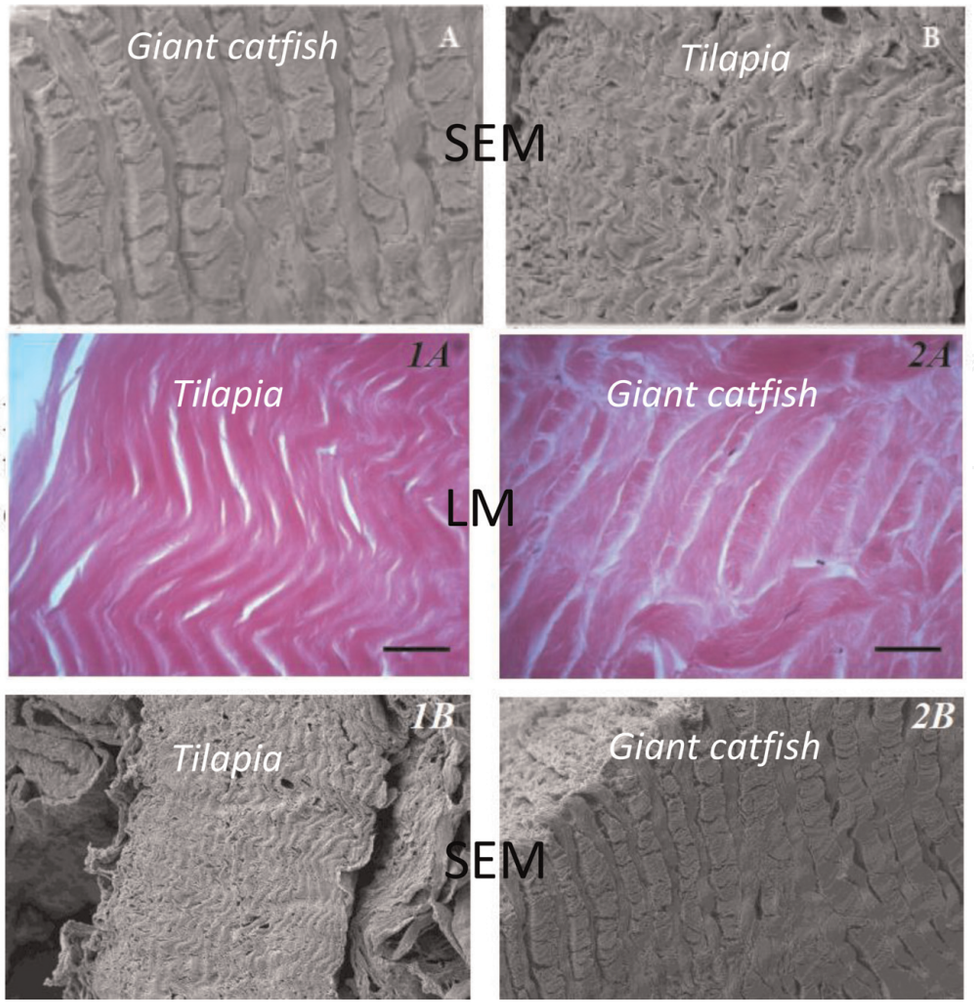


Figure 4.
Microstructure of giant catfish skin (A) and tilapia (B). LM examined at 400× magnifications and SEM magnification of 500× at acceleration voltage of 10 kV. Source: Rawdkuen et al. [16] and Sai-Ut et al. [18].

Chemical compositions of both the fish skin and the gelatins are also presented in **Table 2**. Proteins are the major compound ranging 84–88% (wet wt.). Other components are moisture, ash, and lipid. For the skin of fresh fish, moisture was the major constituent (53–67%). Protein found in GC was higher than TP.

For the functional properties, the results showed that GC and TP are comparable to BF. Protein patterns by using SDS-PAGE showed high band intensity for the α- and β-components in TP, while BF showed the lowest band intensity of the major

Constituents	Gelatin			Fish skin	
	Giant catfish	Tilapia	Bovine	Giant catfish	Tilapia
Yield (%)	19.50 ± 1.87	23.34 ± 2.49	—	—	—
Moisture	3.39 ± 0.43	8.49 ± 0.82	11.38 ± 0.72	53.8 ± 5.3	67.7 ± 0.5
Protein	85.27 ± 0.68	84.28 ± 1.04	88.33 ± 1.10	43.0 ± 0.9	30.6 ± 0.9
Lipid	1.24 ± 0.15	0.45 ± 0.18	0.09 ± 0.01	1.6 ± 0.5	1.1 ± 0.1
Ash	0.17 ± 0.03	0.15 ± 0.09	1.30 ± 0.22	0.3 ± 0.1	2.1 ± 0.4

Sources: Rawdkuen et al. [16] and Sai-Ut et al. [18].

Table 2.
Gelatin yield and proximate composition of fish skin and gelatin from farmed giant catfish, Nile tilapia, and commercial beef skin gelatin. Values are given as mean ± SD from triplicate determinations.

component (**Figure 5**). From the study, it can be concluded that the GC and TP are a prospective source for gelatin production with desirable functionalities. In addition, fish skin gelatin could be more effectively and widely used in food ingredient industries [16].

2.4 Gelatin application

2.4.1 Antimicrobial film

Edible films prepared from different types of biomaterials such as proteins, polysaccharide, lipids, or their blends exhibit different film properties. Among all of these materials, proteins are considered to provide desirable mechanical, gas barrier and transparency properties, as well as high nutrition [32]. Rawdkuen et al. [15] prepared edible films from the gelatin of farmed giant catfish skin (GC), bovine bone gelatin (BB), and their combination (50:50) as depicted in **Figure 6**. The total color difference (ΔE) value of BB films was lower than CG and GC films, respectively. The GC films showed significantly lower values of *L* and *a* but highest in *b* value ($p < 0.05$). When compared with the low-density polyethylene film, GC and BB films showed the highest and lowest total of color differences, respectively. BB film had a lighter color and more yellowness than other films where the highest redness was observed in GC films. The GC films showed the most compact, smooth, and continuous surface without porous structures, which corresponds to the greater properties of films [15].

2.4.2 Antimicrobial and antioxidant films

Some studies regarding to cooperation some natural extracted into protein-based film was summarized in **Table 3**. Natural extracts such as catechin-Kradon, catechin-lysozyme, nisin-catechin, and longan seed extract were used as active ingredients to delay lipid oxidation or retard microbial growth in soybean oil or fresh meat and fish slice. In vitro antibacterial of films incorporated with nisin and/

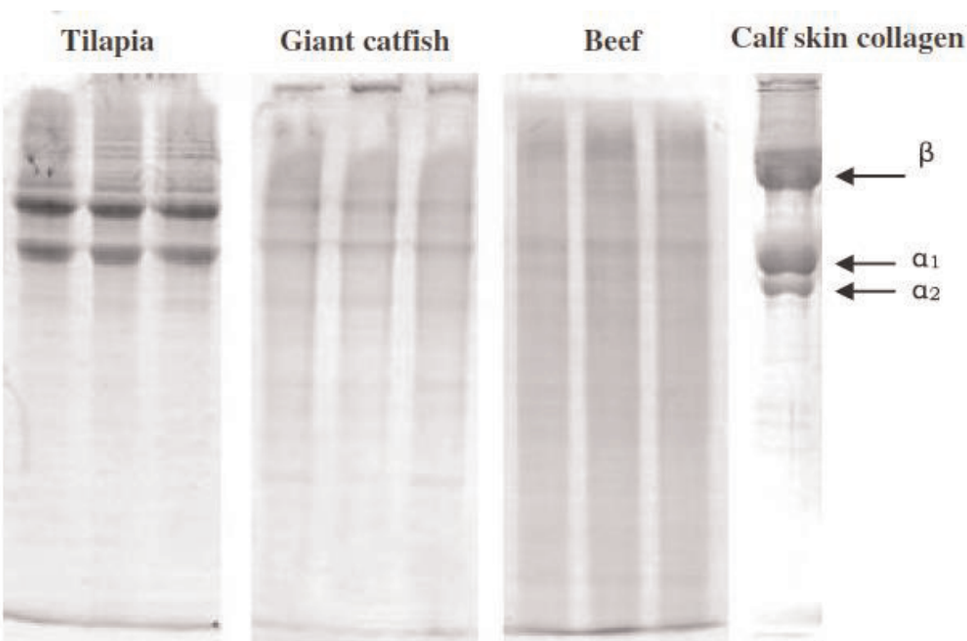


Figure 5.
SDS-PAGE patterns of gelatin from farmed fish skins and commercial beef skin gelatin under nonreducing conditions. Source: Rawdkuen et al. [16].

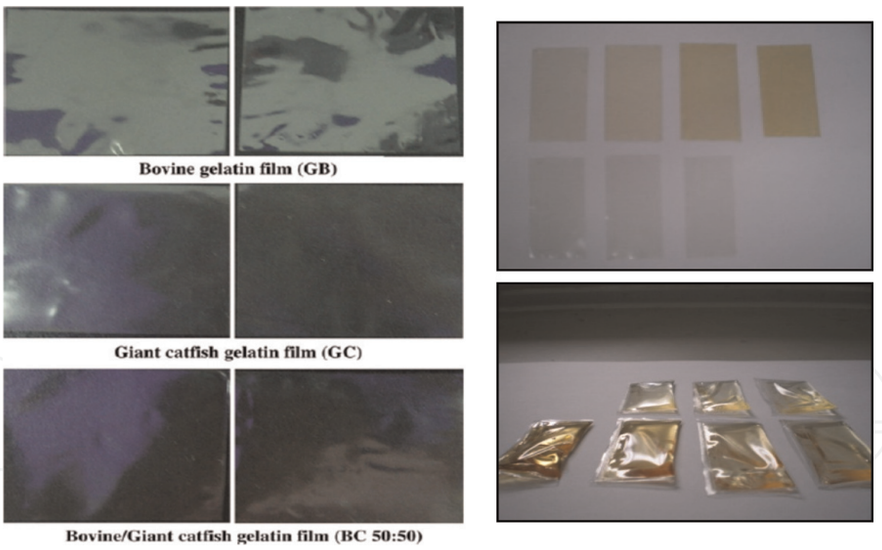


Figure 6.
Surface morphology of gelatin films from bovine bone gelatin, giant catfish skin gelatin and their combination of bovine bone:giant catfish (50:50). Source: Rawdkuen et al. [15].

Protein	Antioxidant/ antimicrobial agents	Sample tested	Loading	Observation	References
Gelatin	Longan seed extract	Soybean oil	50, 100, 300, and 500 ppm	Prevent effect on lipid oxidation 30 days of storage	Vichasilp et al. [37] and Sai-Ut et al. [38]
	Nisin and/or catechin	Minced pork	0.12 g/ 100 g 0.06 g/ 100 g	Retarded lipid oxidation and lower microbial growth rates	Kaewprachu et al. [33]
	Catechin-lysozyme combination (1:1)	Minced pork	0.5 g/ 100 g	Inhibition of lipid oxidation and lower microbial growth rates than those of PVC	Kaewprachu et al. [34]
	Neem extract	Minced beef	0, 0.1, 0.3, and 0.5% (w/v)	Delay oxidative reactions	Putsakum et al. [35]
	Catechin-lysozyme combination	*S. cerevisiae*, *S. aureus*, *E. coli*, and *L. innocua*	0, 0.125, 0.25, and 0.5% (w/v)	Improve mechanical, physicochemical, and antimicrobial properties	Rawdkuen et al. [39]
Fish myofibrillar protein	Catechin and Kradon extract	*Vibrio parahaemolyticus*	0, 3, 6, 9, and 12 mg/ml	Antimicrobial activity	Kaewprachu et al. [40]
	Catechin and Kradon combination extracts	Tuna slice	0.9% (v/v)	Total volatile base nitrogen, peroxide value, and TBARS decreased and lowest growth of psychrophilic bacteria	Kaewprachu et al. [36]

Table 3.
Antioxidant and antimicrobial activity of protein-based films incorporated with antioxidant or antimicrobial agents.

or catechin was investigated by Kaewprachu et al. [33]. Interestingly, gelatin films with nisin and catechin retarded lipid oxidation and microbial growth: the time to reach a total viable count of 10^7 CFUg^{-1} of meat was extended from 1 to 4 days as

illustrated in **Figure 7**. Catechin-lysozyme incorporated gelatin film (CLGF) also showed positive results in preserving the quality and maintaining shelf life of minced pork when compared with that of PVC film [34]. It was found that sample wrapped with CLGF showed less weight loss, less discoloration, and low TBARS content than that wrapped with PVC. Microbial numbers in the sample wrapped with the CLGF (TPC count 4.15 log CFU/g; yeast and mold 2.99 log CFU/g) were lower than those observed in the PVC [34]. The properties of gelatin films incorporated with neem extract (NE; *Azadirachta indica*) as well as catechin-Kradon extract were determined as depicted in **Figure 7**. It was found that gelatin film containing NE is potentially a promising film that could help delay oxidative reactions over a 7-day period in a refrigerated storage (4 ± 1°C) [35].

Besides, catechin-Kradon extract (*Careya sphaerica* Roxb.) was incorporated with fish myofibrillar protein films (FMP) [36]. It was found that decreases in the redness index for the tuna slices were coincidental with increases in metmyoglobin content stored in a refrigerator (4 ± 1°C) for 10 days (**Figure 7**). Moreover, the developed film delayed peroxide value, TBARS, and metmyoglobin contents in Bluefin tuna as well as total volatile base nitrogen and psychrophilic bacteria were

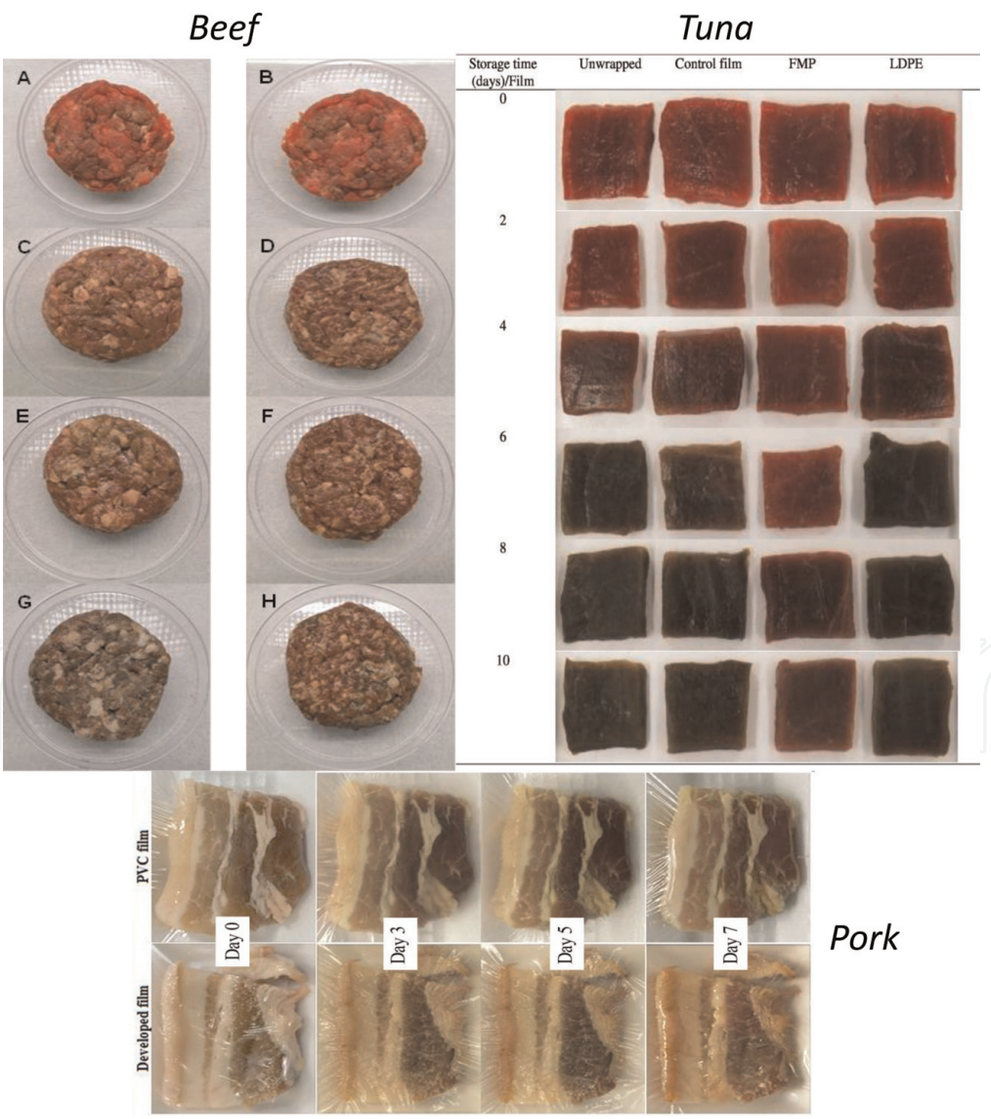


Figure 7.
Quality attribute of meat wrapped with myofibrillar protein film incorporated with natural extract. Sources: Kaewprachu et al. [33], Kaewprachu et al. [34], Kaewprachu et al. [36], and Putsakum et al. [35]. A, C, E, and G represent minced beef wrapped with polyvinyl chloride, PVC and B.D,F, and H represent minced beef wrapped with gelatin film containing 0.3% neem extract, for 0,3,5, and 7 day, respectively.

suppressed Shelf life extension from 4 to 8 days was obtained in FMP sample. FMP showed high efficiency in both antimicrobial and antioxidant activities [36]. Gelatin film incorporated with longan seed extract or BHT could be used as a tool to prolong the shelf life of oily foods [37]. Antioxidant activity of the films incorporated with longan seed extract markedly increased when the storage time increased. LS- or BHT-incorporated films showed the preventive effect on lipid oxidation of soybean oil during storage for 30 days. Longan seed extract film (500 ppm) provided the highest efficacy for lipid oxidation retardation as evidenced by lower conjugated diene values.

2.4.3 Other applications

Apart from film application, gelatin from food processing by-products can be applied to make clarification in juice or protection from syneresis of yogurt. In application as guava juice clarifier study by Widyasari and Rawdkuen [31], the addition of gelatin at different concentrations showed different results, where the highest value of turbidity was observed from tilapia gelatin at concentration of 1% (w/v) (382 FTU). Furthermore, the lowest turbidity value is purchased at 0.16% (w/v) from chicken feet gelatin (108 FTU). On the other hand, the syneresis of yogurt after centrifugation at 4°C ranged from 0.0 to 71.49%, where the lowest syneresis index was obtained at 1% (w/v) level of the added gelatin from chicken feet and tilapia gelatin.

2.5 Fish bone

Fish bone weighs as high as 52–60% of fish body weight. Proximate composition of giant catfish bone was shown in **Table 4**. Fish bones can serve as a raw material for the production of high value-added compounds that can be used in various sectors including agrochemical, biomedical, food, and pharmaceutical industries [23]. Those bones can serve as a promising source of calcium. The bone is composed of 34–36% calcium, based on total ash content, and is also rich in collagen and chondroitin. Additionally, fish bone is the source of hydroxyapatite (HA) known as $(Ca^{2+})_{10-x}(H_3O^+)_{2-x}(PO_4{}^{3-})_6(OH^-)_2$. Those bones could be converted to biocalcium (BC) powder, which is derived from bio-mineralized or a naturally produced resource [24].

Permpoon et al. [25] worked to develop food seasoning powder supplemented with fish bone as depicted in **Figure 8**. Different concentrations of fish bone powder (0–3%, w/w) were added in those recipes with and without monosodium glutamate (MSG). The content of minerals was shown in **Table 5**. The highest score of overall acceptance was 6.40 in the sample supplemented with 0.5% fish bone powder containing MSG. High calcium, phosphorus, and sodium were predominant mineral content in the sample supplemented with fish bone [25].

Composition	Percentage
Moisture	6.79 ± 0.09
Protein	28.14 ± 0.10
Fat	8.61 ± 0.38
Minerals	50.09 ± 0.19

Source: Terzioğlu et al. [23].

Table 4.
Proximate composition of giant catfish bone.

Figure 8.
Food seasoning powder supplemented with fish bone. Source: Permpoon et al. [25].

Component	Conditions*		
	Control	1% Fish bone	Commercial
Calcium**	94.48 ± 8.65[b]	1637.50 ± 252.44[a]	120.25 ± 5.02[b]
Potassium	2088.50 ± 41.72[a]	2115.50 ± 85.56[a]	2122.50 ± 136.47[a]
Phosphorus	493.90 ± 23.19[b]	1338.00 ± 0.69[a]	683.70 ± 32.24[b]
Magnesium	60.62 ± 2.98[c]	80.38 ± 0.69[b]	92.52 ± 3.96[a]
Sodium	182,550 ± 6151[a]	185,050 ± 19,728[a]	171,800 ± 16,687[a]

Source: Permpoon et al. [25]
**Values are represented as mean ± SD (n = 2). Different lowercase letters in the same row indicate significant difference ($p < 0.05$).*
***mg/kg*

Table 5.
Mineral content in food seasoning supplemented with fish bone values which are presented as mean ± SD (n = 2). Different lowercase letters in the same row indicate significant difference ($p < 0.05$).

2.5.1 Fish bone as calcium and phosphorus source

Fish bones have high calcium content, and large quantities of this raw material are available as a waste from the fishery industry. Previously, emphasis has been done on producing high-quality products from fish by-products by the use of bacterial proteases [41]. Biocalcium powder from the bones of precooked skipjack tuna was produced and characterized compared with calcined bone (CB) powder. Higher calcium (40.35%) and phosphorous (15.28%) contents were found in the CB powder than BC powder (26.91 and 12.63%, respectively). BC powder had a low abundance of volatile compounds, including aldehydes, alcohols, and ketones. Precooked skipjack tuna bone could be used as a raw material for preparing BC powder, which has a different composition than CB powder. The BC powder was still composed of collagenous protein. BC had a low abundance of odorous compounds, whereas the CB powder had a negligible amount of volatiles. Precooked skipjack

tuna is considered a promising source for BC production due to its abundance. BC with improved color and odor along with increased solubility in gastrointestinal tract can be used as an alternative calcium supplement to tackle the inadequate intake of dietary calcium.

3. Plant processing by-products

The waste portions such as the peel, core, stem, and crown were 29–40, 9–10, 2–5, and 2–4% (w/w), respectively (**Figures 9** and **10** and **Table 6**) [42–45]. From the last decade, the use of plant extracts as a source of bioactive components (phytochemicals) has gained wide attention against synthetic antibiotic drugs. Pineapple peels and cores account for about 40% of the whole fruit, and they are largely wasted after fresh-cut processing. Papaya fruit is the green fruit which is widely used in Thai cuisine and famously in papaya salad. As the consumption increases, large quantities of papaya peels as by-products are left [42]. Usually, the peels are occasionally used for animal feed or disposed, which produce phytopathogens, then cause ecological problems, and pose risks to human health. These wastes are occasionally utilized as fertilizers or animal feed, yet they have very low economic value. Therefore, ways to utilize these residue wastes have become an important focus for research and development, recognizing that a systematic reduction in waste disposal is beneficial both economically and ecologically. Thus, fruit waste extracts are suitable for enhancing the nutritional and antioxidant properties in food, and moreover, they can also be applied in cosmetics and the nutraceutical and pharmaceutical industries [46]. Therefore, the utilization these waste products have become an important task for research and development. Systematically reducing waste and putting it to other uses is profitable both economically and ecologically speaking [37, 38].

3.1 Fruit peel

Peel is the outermost covering of fruits and not commonly consumed. Pineapple and papaya peels are found to have potential uses as raw materials that could be converted into value-added products, especially as sources for bioactive compound extraction. The utilization of fruit peels as a source of bioactive compounds, especially in proteolytic enzymes extraction means [44, 47].

3.2 Enzyme extraction and application

The isolation and characterization of bromelain extract (BE) from the wastes of *Nang Lae* and *Phu Lae* pineapple cultivars (economical fruits of Chiang Rai province, Thailand) were investigated by Ketnawa et al. [43] and Ketnawa et al. [44].

Figure 9.
Peel from agriculture products, pineapple, papaya, and mango (left to right). Source: Ketnawa et al. [42].

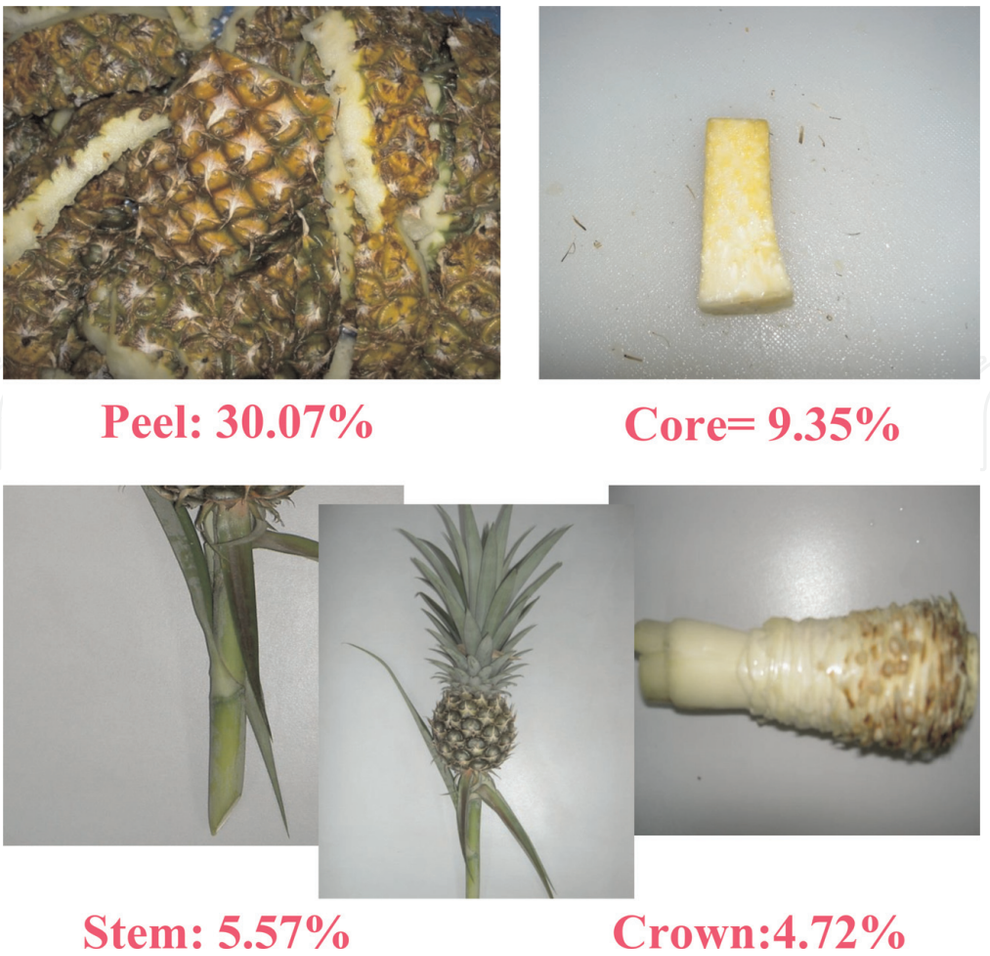


Figure 10.
By-products from pineapple fruits. Source: Ketnawa et al. [44].

Part of pineapple	*Nang Lae*		*Phu Lae*	
	Weight (g)	**% (w/w)**	**Weight (g)**	**% (w/w)**
Peel	143.40	30.09	159.86	42.20
Core	44.61	9.36	40.60	10.72
Stem	26.55	5.57	9.26	2.44
Crown	22.48	4.72	10.20	2.69
Flesh	239.86	50.33	158.94	41.95
Total	476.54	100.00	378.86	100.00

Source: Ketnawa et al. [43].

Table 6.
Proportion of pineapple fruits of Nang Lae and Phu Lae cultivars.

The extract of crown from both cultivars gave the highest proteolytic activity and protein contents, while the extract from the stem exhibited the lowest values. Bromelain is a major enzyme with the MW ~ 28 kDa. This study founded that there is much added value into local Thailand pineapple wastes because of bromelain extraction [44]. Chaiwut et al. [47] have reported that they obtained a substantial extraction of proteases from papaya peels. Tyrosinase inhibitor enzyme was extracted from mango seed kernel [46, 48].

Bromelain has been used commercially in the food industry, in certain cosmetics, and in dietary supplements. It is used for meat tenderizing, brewing, and baking, as well as for the production of protein hydrolysates [42]. Toughness is one of the most common quality characteristics of meat and can be subdivided into actomyosin toughness and background toughness. The former is attributable to changes in myofibrillar proteins, whereas the latter is attributable to connective tissues. Treatment by proteolytic enzymes is a popular method for meat tenderization. Proteolytic enzymes derived from plants such as papain, bromelain, and ficin have been widely used as meat tenderizers in most parts of the world. Ketnawa and Rawdkuen [45] used bromelain extract obtained from the top phase of an aqueous two-phase system (18% PEG-6000 + 17% $MgSO_4$) to tender the muscle foods with different concentrations of BE (0–20%, (w/w)) for 1 h. A reduction of meat firmness and toughness was observed in all samples when compared to the control. Electrophoretic patterns revealed extensive proteolysis and a reduction in number and intensity of the protein bands in all of the treated samples. The results showed that the bromelain extract could be used as an effective meat tenderizer. In similar study of Ketnawa et al. [43], the effects of the bromelain extract on the protein patterns of beef, chicken, and squid muscles were also determined as depicted in **Figure 11**. Trichloroacetic acid-soluble peptide content of all the treated muscles increased when the amount of bromelain extract increased. Decrease in myosin

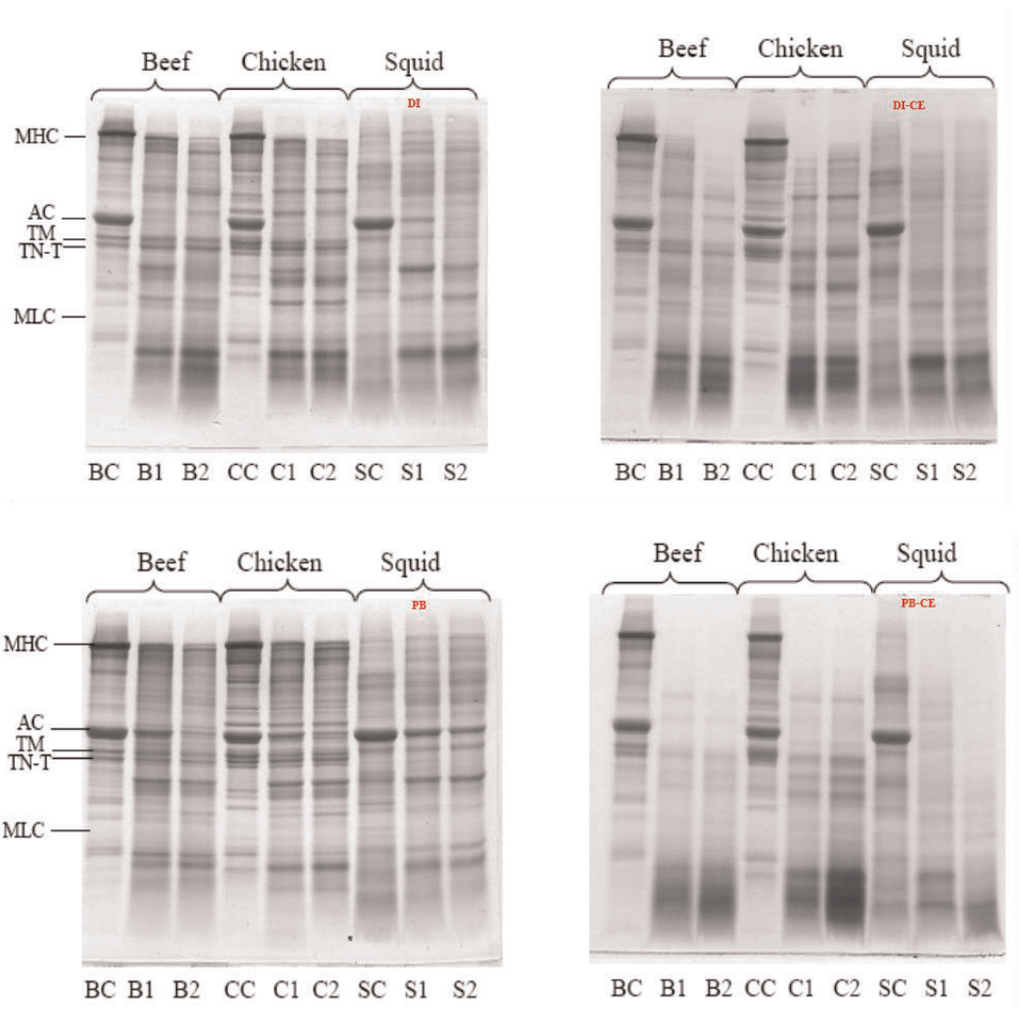


Figure 11.
SDS-PAGE patterns of muscles treated with bromelain from pineapple peels. BC, CC, and SC, controls without extract: B, beef; C, chicken; and S, squid. The numbers 1 and 2 indicate the amount of extract, 1 and 2 mL, respectively. MHC, myosin heavy chain; AC, actin; TM, tropomyosin; TN-T, troponin-T; and MLC, myosin light chain. Source: Ketnawa et al. [43].

heavy chains and actin was observed in all the muscle types when bromelain extract was used [43]. The technology for applying this enzyme is cheaply available and can be exploited at the household or industrial level for tenderizing meat, and it can be used as an alternative to chemical tenderizers or other plant proteases.

3.3 Fruit seed and kernel

Recent studies have shown that fruit's waste parts like mango seed contain a noteworthy amount of bioactive component of therapeutic worth [48, 49]. These biologically active components include mangiferin, flavonoids, catechin, phenolic acids, gallic acid, and gallic acid derivatives as shown in **Figure 12**. The therapeutic importance of these compounds have evaluated through in-vitro and minimal preclinically, but there is a need for proper preclinical trials and afterward clinical trials for health claims and health benefits. The industrial processing of mangoes produces several million tons of waste from their peels and seeds at various stages that cause for a major disposal problem and effect to the environment. The seed alone makes up about 20% of the whole fruit, with 45–78% of the seed being the seed kernel [49, 50]. During processing of mango, by-products such as peel and kernel are generated.

The major polyphenols present in mangos that act as a source of natural antioxidants (**Figure 12**) are as follows: mangiferin, catechin, quercetin, kaempferol, cinnamic acids, tanins, etc. The extract of the mango peels and the seed kernels also has a great deal of tyrosinase inhibitor, antioxidant activity, and chelating activity [46, 49, 51].

3.3.1 Bioactive compound extraction

To enhance the efficiency of the extraction process, optimization the extraction parameters: the liquid/solid ratio, the ethanol concentration, and the extraction time are consideration. The study describes the optimization of polyphenol extraction from mango seed kernels by using response surface methodology (RSM) (**Figure 13**). Sai-Ut et al. [46] applied RSM to optimize the ethanolic extraction of polyphenols from mango peels. Nam-Dokmai peel (NDP) showed significantly higher phenolic content and tyrosinase inhibitor activity than that of the Tong-Dam peel (TDP). The optimal condition that maximized the extraction yields, EPC, and the antioxidant activities for NDP were ethanol proportion of 49%, a temperature of 61°C, and an extraction time of 221 min, whereas the optimized condition that maximized the extraction yields, TPC, and antioxidant activities from mango

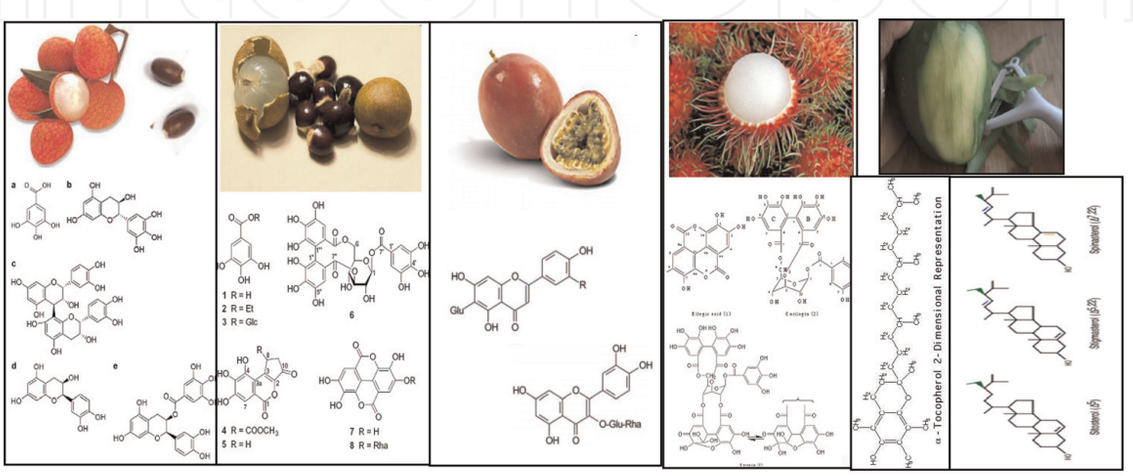
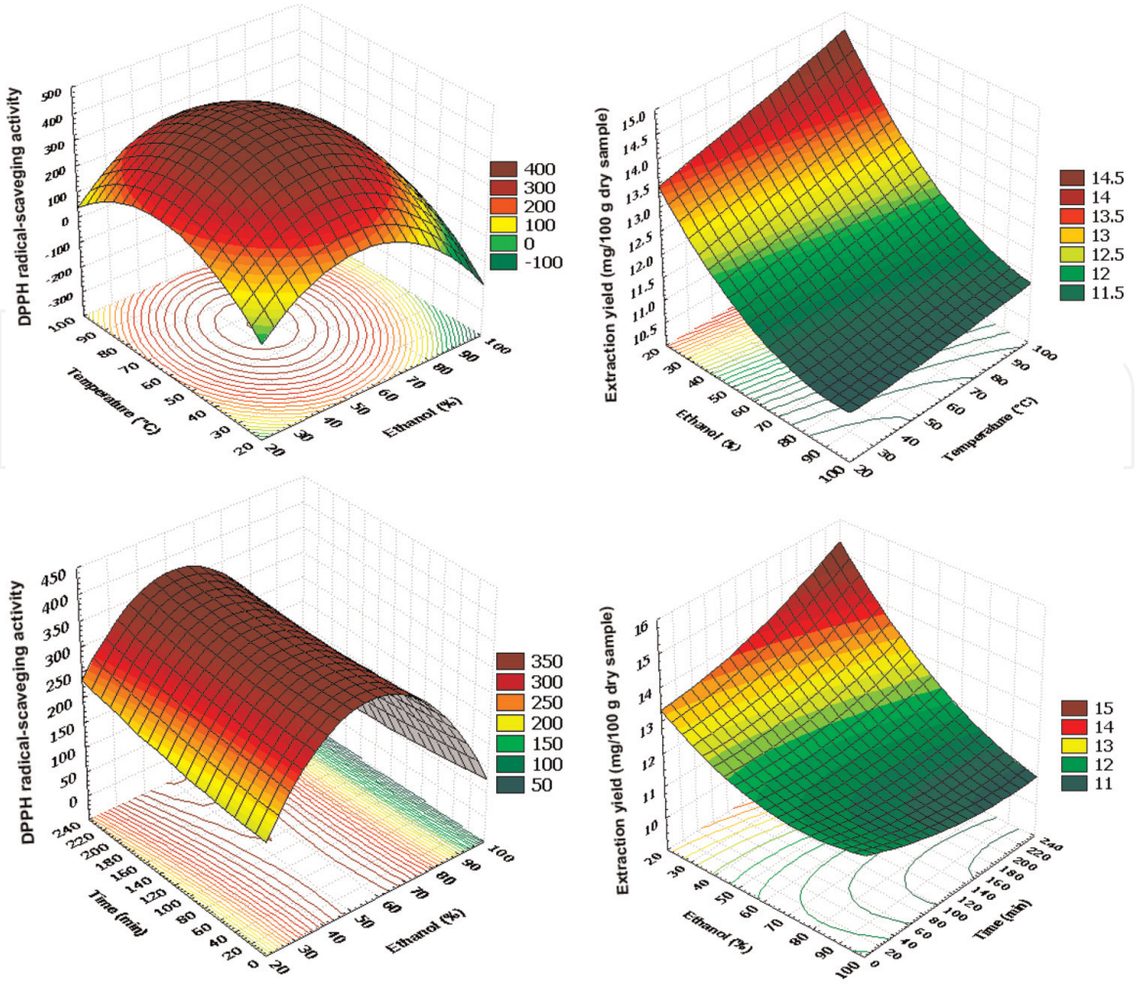


Figure 12.
Chemical structures of some phenolic compounds found in plant by-products.



Regression coefficients of predicted models & response surface model plot showing the effects of temperature and ethanol proportion and effects of time & ethanol proportion on EPC & DPPH (mg GAE/100g dry sample) from lychee seeds

Regression coefficients of predicted models & response surface model plot showing the effects of temperature and ethanol proportion and effects of time & ethanol proportion on extraction yield (mg /100g dry sample) from rambutan seeds extracts

Figure 13.
Response surface methodology to optimize the extraction of polyphenol compounds from plant wastes. Sources: Rawdkuen et al. [48] Sai-Ut et al. [46].

Tong-Dam kernel (TDK) was an ethanol concentration of 62%, a temperature of 63°C, and a duration of 150 min with a fixed ratio of 1:30 solid-liquid [48].

3.4 Legume/grain/defatted seed flour/rice bran

Sacha inchi is widely utilized as a raw material in the edible oil industry not only in South Africa but also in Asian country. The de-oiled meals contain high amounts of proteins, which makes it highly desirable for industrial use as value-added products. The proximate compositions of sacha inchi used in this study included protein 459, carbohydrate 361, fat 67, ash 59, crude fiber 58, and moisture 53 g/kg (wb). A comparative study of pressed cake made from tea and sacha inchi seeds was performed. Sacha inchi seeds contained the largest amount of protein (62.07%) and tea seeds contained the largest amount of carbohydrates (82.68%) as shown in **Figure 14**. Lysine, leucine, histidine, and phenylalanine were the main essential amino acids. Protein patterns by using SDS-PAGE showed that the main protein component had MWs of 35–63 for sacha inchi and 11–20 kDa for tea seeds. In addition, it contained glycoprotein with a MW of 35 kDa. Both pressed cakes

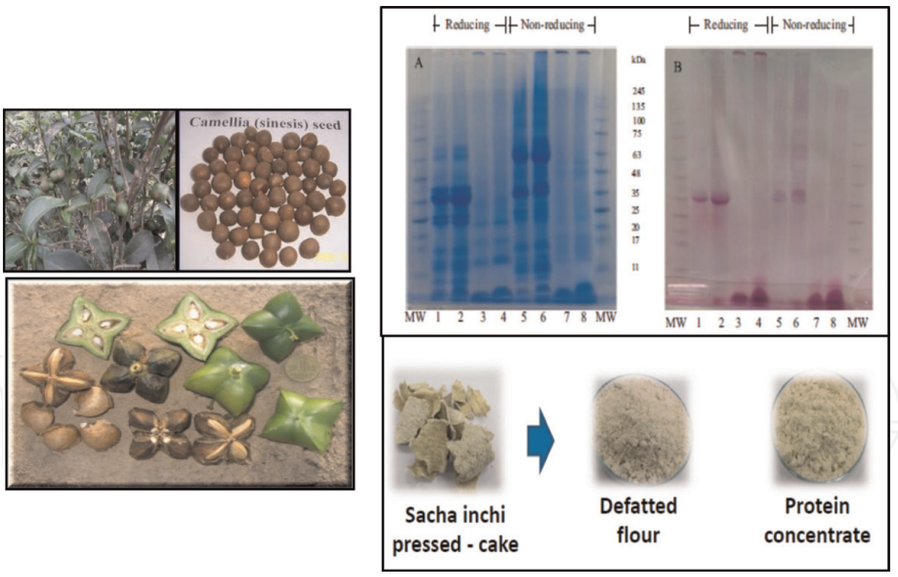


Figure 14.
Some utilization of pressed cake from sacha inchi and tea seeds. Source: Rawdkuen et al. [53]. A and B represent Electrophoresis patterns of protein and glycoprotein of pressed-cake from tea and sacha inchi seeds, respectively.

showed good sources for bioactive compounds with high antioxidant activities. Therefore, the chemical properties of the pressed cakes indicate that this by-product of oil extraction is a good supplement to functional food ingredients. Nutritional factors such as essential amino acids, essential fatty acids, dietary fiber, and mineral content suggest that the pressed cake with sacha inchi seeds could be a useful ingredient for human consumption [52, 53].

3.4.1 Protein hydrolysate preparation from sacha inchi pressed cake

Protein concentrate (PC) was prepared (**Figure 15**), and their hydrolysate (PH) was hydrolyzed by crude papain and Calotropis proteases according to Rawdkuen et al. [53]. PC was hydrolyzed by crude papain (PH-P), and Calotropis proteases (PH-C) had a degree of hydrolysis (DH) of 2.7 and 11.2%, respectively. PH-P contained a higher amount of essential amino acids (474 g/kg) than PH-C (410 g/kg). The protein patterns of PC and PHs by using SDS-PAGE showed the molecular weights between <8 and 57 kDa. DPPH scavenging activity and FRAP assay showed a rising PH when the DH was increased. Sacha inchi protein hydrolysates can be produced in a cost-effective way by using natural crude enzyme extracts.

Some applications of protein concentrate in food model system were performed in pork sausage and fish finger (**Figure 16**). The results showed that when the concentration of protein concentrate increased, the textural properties of the sausage were improved. In addition, with fried fish finger can extent it shelf life when protein hydrolysates was added when compared with the control treatment. Both hydrolysates showed effective bioactivities as well as high nutritional value (Lys, Phe, and Tyr) after in vitro gastrointestinal digestion. Thus, crude papain extracted from papaya peel and *Calotropis proteases* from latex can be used as alternative natural enzymes for production of protein hydrolysates by using sacha inchi meal as a starting material [53].

3.4.2 Extraction and isolation of protein form rice bran

A plenty of rice bran are produced as by-products during rice milling process nowadays following the demand in production and consumption. After rice milling

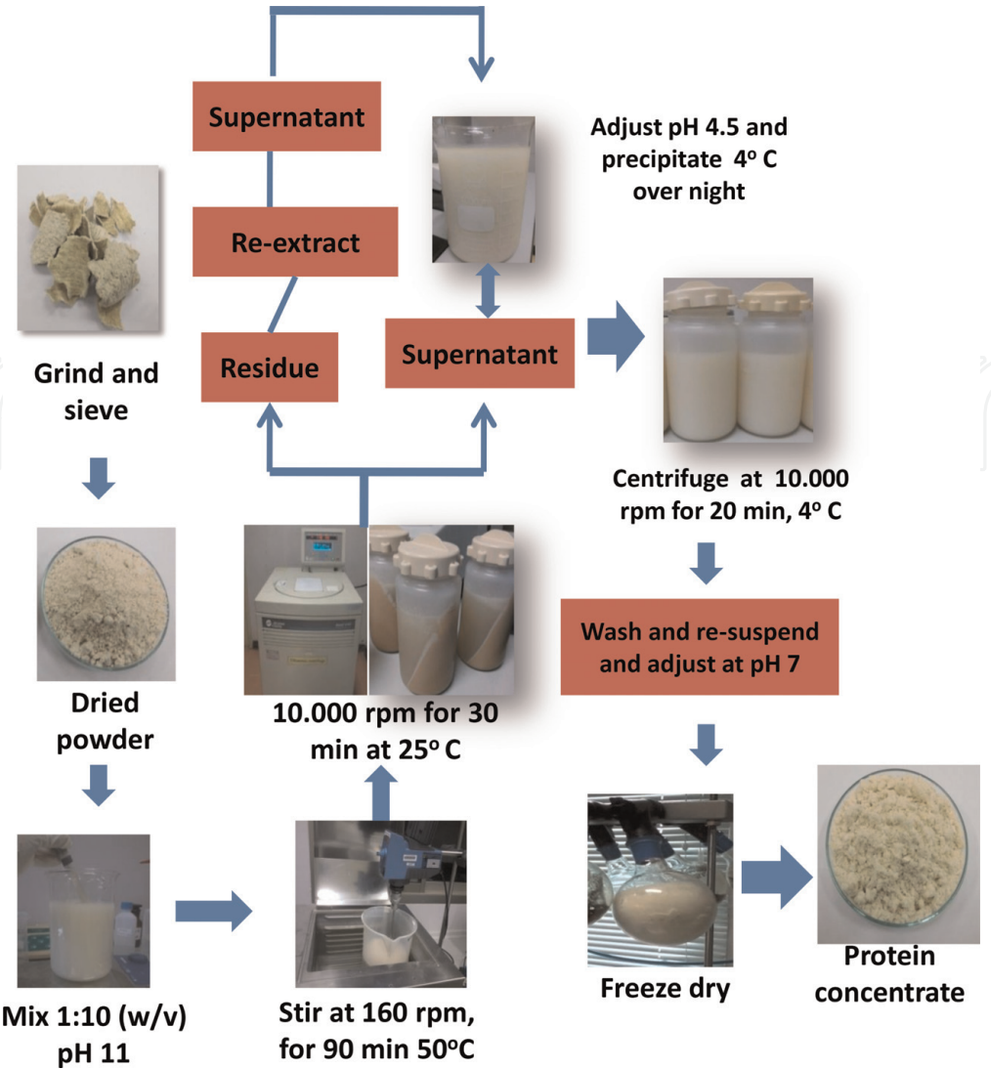


Figure 15.
Preparation of protein concentrate from pressed cake sacha inchi. Source: Rawdkuen et al. [53].

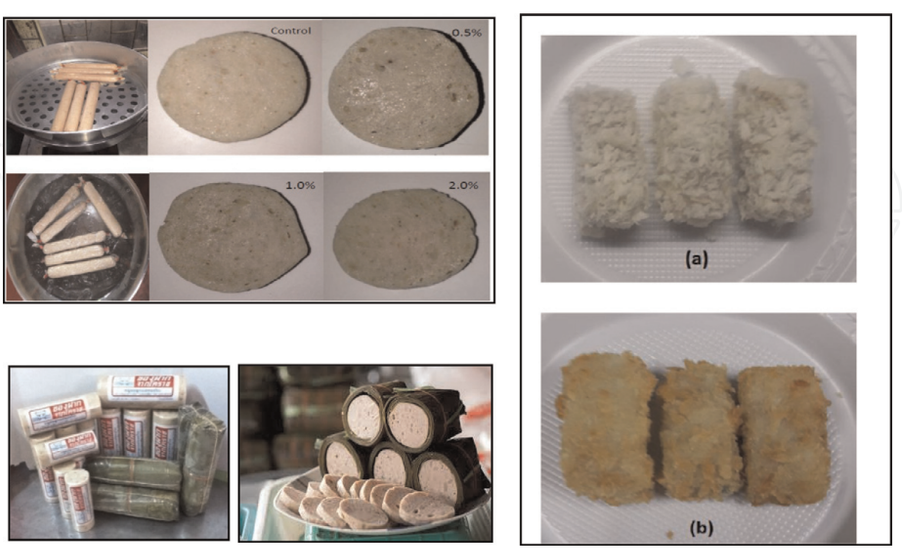


Figure 16.
Application of protein concentrate from sacha inchi defatted meal in fish fingers. Source: Rawdkuen et al. [53]. (a) and (b) represent fish finger contained sacha inchi protein concentrate before and after frying, respectively.

process, 70% rice is obtained, while rice hull (20%), rice bran (8%), and rice germ (2%) are the by-products. The major portion of this is used as animal feed or discarded as waste material [54]. However, rice bran is attracting attention from

researchers because it is an alternative and economic source of plant-based hypoallergenic and high-quality protein [54, 55]. Besides, rice bran is a rich source of protein, fat, carbohydrate, vitamins, minerals, dietary fiber, and bioactive compound antioxidants. Furthermore, the chemical, functional, and biofunction properties of rice protein seem superior to other proteins such as soya flake, potato starch, peanut, sorghum, kidney bean, and groundnut [55, 56]. Thus, rice bran has a strong potential to extract certain bioactive compounds, that is, protein concentrate, protein hydrolysates, active peptides, and utilization of those compounds, that is, film production (**Figure 17**).

Rice bran fraction is the main source of protein in rice grain. The protein content ranges from 10 to 16% (W/W) depending on its cultivars [57]. Rice bran is an economic source of high-quality plant-based protein that can exhibit an excellent functional properties and interesting bio-functions. Rice bran protein is suggested as one of the important plant-based proteins that can be applied or used as ingredient in many products such as infant food, meat ball, noodles, biscuit, breads, and gluten-free (GF) products. Moreover, its hydrolysate form has a potential to be

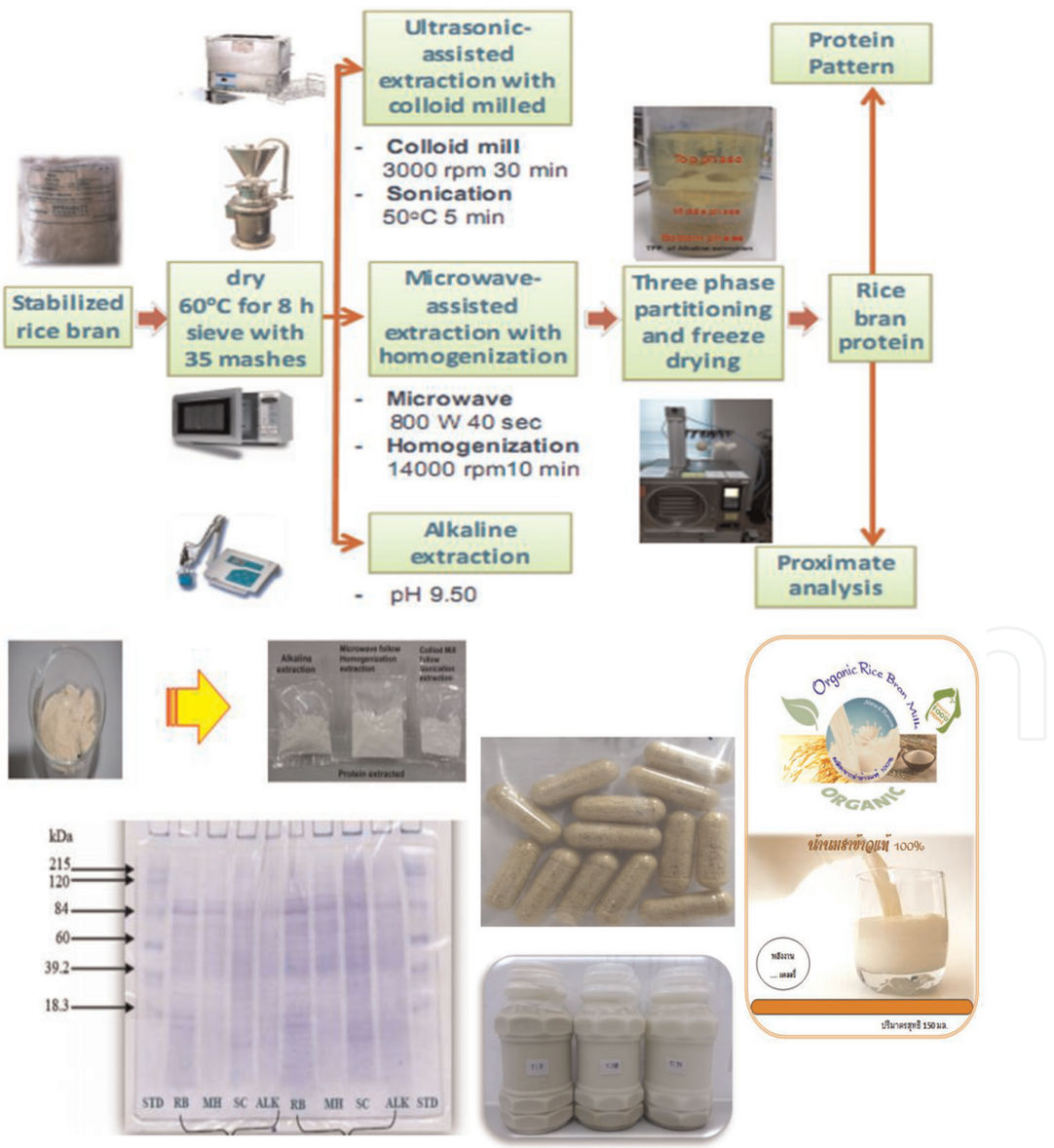


Figure 17.
Different methods for extraction of protein from rice bran. Sources: Phongthai et al. [62], Phongthai et al. [63], and Phongthai et al. [64].

applied in nutraceutical products and also cosmetic goods [58]. Thus, the several methods to extract protein from rice bran fraction are of interest and developed through physical, chemical, and enzymatic treatments.

Different extraction methods both conventional extraction such as solvent extraction [59, 60], alkaline extraction [57, 58] as well as innovative ones such as supercritical fluid [61], microwave [62], ultra-sonication [63] and enzymatic extraction [64] are used to extract protein from rice bran. Conventional method like alkaline extraction is the most common method for extracting protein from plant materials due to its simplicity and low cost. However, severe alkaline conditions negatively affect the nutritional and functional properties of the protein. This process also requires a long time for extraction and consumes large volumes of buffer. Moreover, exposing protein to severe alkaline conditions also affects the nutritional and functional properties of protein [65]. Therefore, other methods such as physical, that is, microwave- and ultrasonic-assisted extraction, as well as enzymatic methods are increasingly being considered as alternative methods.

Nowadays, microwave and ultrasonic are commonly applicable methods in food preparation, especially for solid-liquid extraction. It is high reproducibility in a shorter time, convenience, and less solvent consumption. Response surface methodology could be used instead of other methods that test only one variable at a time, which is time-consuming and not cost-effective. It also provides insight into the interactions of the variables and calculates the optimal response with a limited number of experiments. The integration of ultrasonic and RSM is challenging but beneficial because it could create a systematic, practical, and economical method for rice bran protein extraction.

Phongthai et al. [63] studied of extraction of protein from organic rice bran by three different methods such as alkaline [58], microwave [62], ultra-sonicated extraction [63], enzyme-assisted extraction (EAE) of rice bran protein [64] and then that protein concentrate was isolated by three-phase partitioning (TPP) techniques [66] or utilization of protein concentrate as raw material for production of protein hydrolysate [58], gluten free bread [58] and pasta [67] as depicted **Figure 18**.

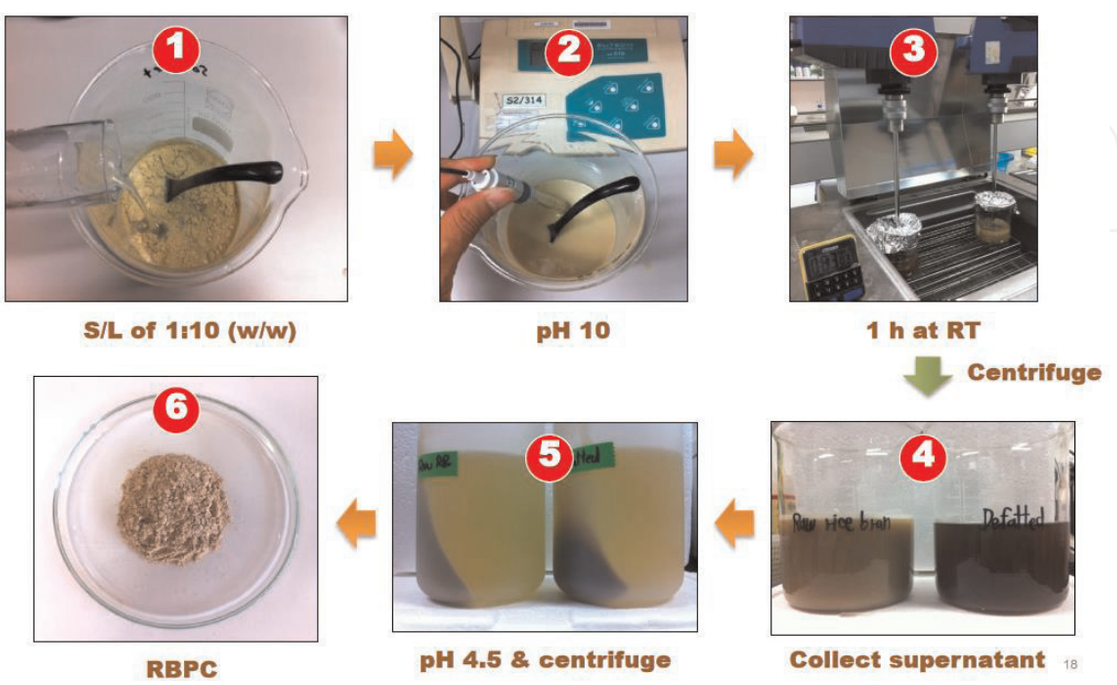


Figure 18.
Rice barn protein extraction by conventional alkaline extraction method. Source: Phongthai et al. [58].

3.4.2.1 Alkaline extraction (ALK)

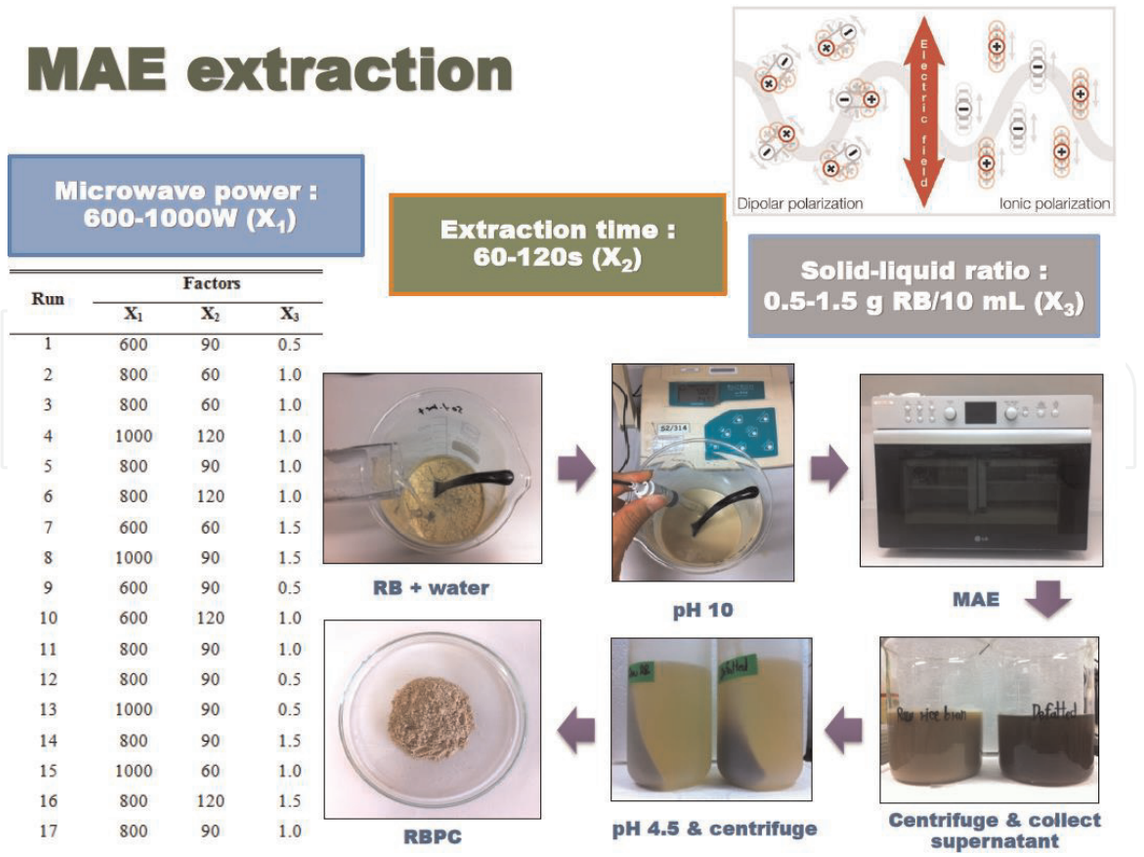
Alkaline extraction is the most common method for extracting protein from plant materials due to its simplicity and low cost. Phongthai et al. [58] studied the protein extraction from organic rice bran using alkaline extraction method which is shown in **Figure 18**. For alkaline extraction, DFRB was dispersed in distilled water (1:10, w/v), and the pH was adjusted to 10 using 3 M sodium carbonate. The mixture was stirred using a pilot-scale stirrer at a temperature of 50°C for 1 h. After centrifugation at 10,000 × *g* for 10 min, the supernatant was collected and adjusted to pH 4.5 using 3 M citric acid and centrifuged at the same conditions. The dried powder is referred to as rice bran protein concentrate (RBPC). From the study, the pilot scale gained RBPC showed a yield of 7.44 g/100 g based on DFRB weight. Compared with lab scale extraction, the used pilot scale procedure gained much higher yields, 1.6–2.7-fold more protein. The main composition of RBPC was protein with 68.07 ± 0.54% dm, which can be labeled as protein concentrate due to the protein content of more than 60% [58]. In addition, two step statistical design (1) fractional factorial design 2^{4-1} (independent variables: X_1 (pH: 8, 9, 10), X_2 (temperature: 25, 35, and 45°C), X_3 (stirring speed: 80, 100, and 120 rpm) and X4 (stirring time: 60, 120, and 180 min) and (2) central composite rotatable design with variables X_1 (temperature: 35, 38, 45, 52, and 55°C) and X_2 (stirring time: 120, 146, 210, 274, and 300 min) was used to extract rice bran protein [57]. However, with this experimental design provided only 48.53% of protein content at pH 10.0, 80 rpm, 300 min of stirring time, and 52°C, with an extraction yield of 34.51%.

3.4.2.2 Microwave-assisted extraction (MAE)

Rice bran was extracted for protein by MAE using RSM, and a three-level three-factor Box-Behnken design was chosen to evaluate the effect of microwave power (X_1), extraction time (X_2), and solid-liquid ratio (X_3) as shown in **Figure 19** [62]. It was found that the optimal condition was 1000 W of microwave power, 90 s of extraction time, and a solid-liquid ratio of 0.89 g rice bran/10 mL of distilled water. The protein yield of MAE showed higher than that of ALK by about 1.54-fold [62]. The ratio of rice bran per water as 1:10 w/v and the temperature controlled at 40°C by a sensor inserted in a control vessel during 90 s of process time, generating a power range of 350–400 W, showed 11% yield and 75% protein content [68]. In the study of Bandyopadhyay et al. [69], the same ration of rice bran solution sample (100 mL) was simultaneously exposed at a frequency of 2450 MHz and operated at 800 W. The result showed that only 40 s of microwave treatment could give the protein recovery of 78.4% as against 28.9% after 1 min for conventional boiling.

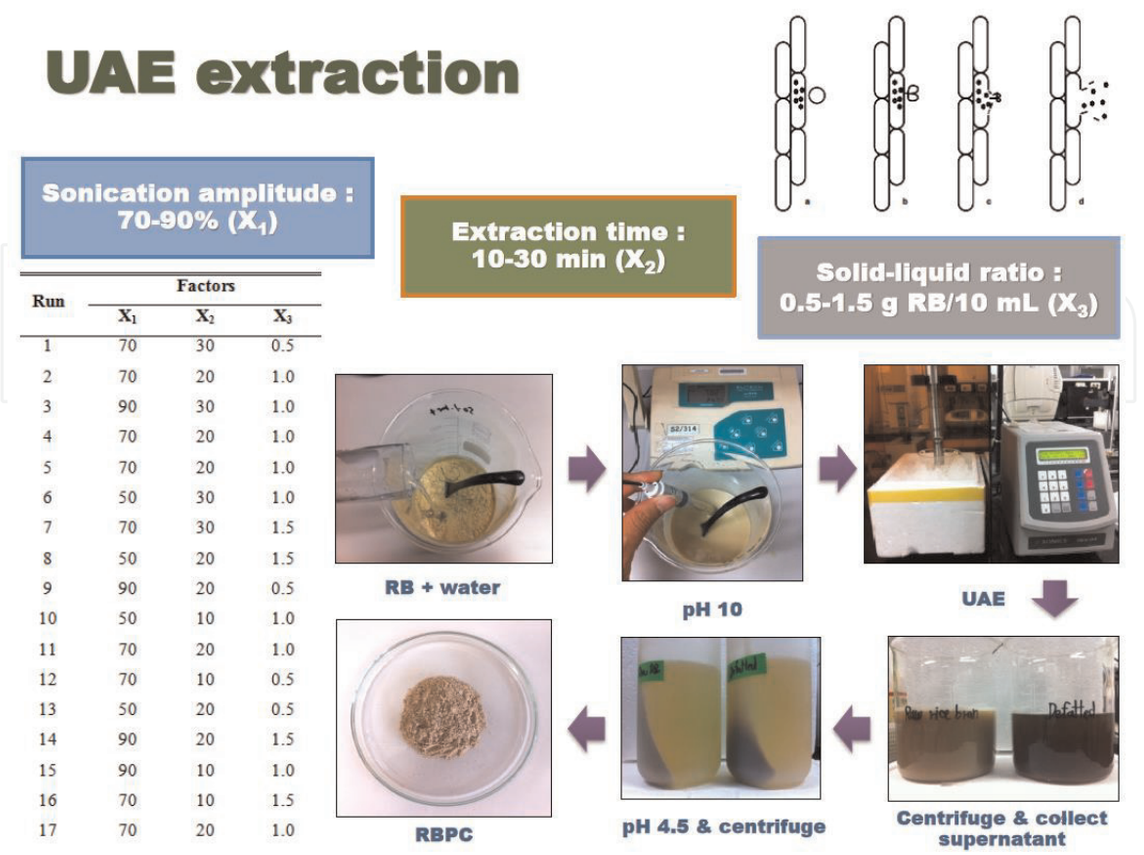
3.4.2.3 Ultrasonic-assisted extraction (UAE)

Another alternative method like UAE was used by Phongthai et al. [63] to study the condition of sonication amplitude (X_1, 50–90%), extraction time (X_2, 10–30 min), and solid-liquid ratio (X_3, 0.5–1.5 g RB/10 mL) for the extraction of protein, as depicted in **Figure 20**. The optimal condition for rice bran protein production was 76% sonication amplitude, 18 min extraction, and 0.99 g/10 mL solid-liquid ratio, which gave a protein yield of 4.73 ± 0.03%. It was found that the sonication amplitude and extraction time influenced a protein yield during UAE. The optimized UAE was a more effective method than alkaline extraction method [63]. The ratio of rice bran per water as 1:10 w/v showed 11.40% yield and 73.80% protein content when using UAE [68].



Run	Factors		
	X_1	X_2	X_3
1	600	90	0.5
2	800	60	1.0
3	800	60	1.0
4	1000	120	1.0
5	800	90	1.0
6	800	120	1.0
7	600	60	1.5
8	1000	90	1.5
9	600	90	0.5
10	600	120	1.0
11	800	90	1.0
12	800	90	0.5
13	1000	90	0.5
14	800	90	1.5
15	1000	60	1.0
16	800	120	1.5
17	800	90	1.0

Figure 19.
Rice bran protein extraction by microwave-assisted extraction method. Source: Phongthai et al. [62].



Run	Factors		
	X_1	X_2	X_3
1	70	30	0.5
2	70	20	1.0
3	90	30	1.0
4	70	20	1.0
5	70	20	1.0
6	50	30	1.0
7	70	30	1.5
8	50	20	1.5
9	90	20	0.5
10	50	10	1.0
11	70	20	1.0
12	70	10	0.5
13	50	20	0.5
14	90	20	1.5
15	90	10	1.0
16	70	10	1.5
17	70	20	1.0

Figure 20.
Rice barn protein extraction by ultrasonic-assisted extraction method. Source: Phongthai et al. [63].

3.4.2.4 Enzyme-assisted extraction (EAE)

Phongthai et al. [64] studied the single study of variables, including solid-liquid ratio (0.5–2 g DFRB/10 mL), extraction time (2 h), and enzyme concentration (5000 units) in rice bran protein extraction. The results showed that the use of solid-liquid ratio at 0.5:10 and 1.0:10 for rice bran protein extraction gave comparable protein recovery and protein yield, reaching maximum values of 12.06 and 2.78%, respectively (extraction time 3 h and enzyme concentration 2500 U). The protein recovery and protein yield increased to 14.20–14.75% and 3.28–3.35%, respectively, when the extraction time extended to 4–5 h. These values increased as the concentration of enzymes increased. Protein recovery and protein yield reached the maximum values of 16.69 ± 0.58% and 3.70 ± 0.12%, respectively, when 10,000 U of enzyme was used [64]. The yield of RBPC prepared by alkaline extraction followed by acidic precipitation is 10.2%, which is further increased to 14.5 and 22.4% by papain- and Viscozyme-assisted extraction, respectively [69].

3.4.3 Application of isolated rice bran protein

3.4.3.1 Bread preparation

Gluten-free-based products have been studied for several years especially for quality improvement by enriching with proteins. Besides, the allergenic character of common protein source like egg albumin is a limitation factor. Phongthai et al. [58] studied the replacement of egg albumin with rice bran protein concentrate which is a nonallergy protein in order to improve the quality of GF bread. The obtained RBPC was composed of 68.07% protein (dry basis) preparation from alkaline-acid extraction technique. The summary of GF breads enriched with different levels of egg albumin and RBPC is depicted in **Figure 21**. The addition of RBPC had strongly influenced the rheological properties, especially elastic modulus (G′) of GF batters during oscillation and the relative elasticity of final GF breads. Breads enriched with 2% RBPC and a combination of 1% egg albumin and 1% RBPC had the highest

Figure 21.
GF breads enriched with different levels of egg albumin and RBPC preparation. Source: Phongthai et al. [58].

specific volume. The properties of GF bread was improved in terms of specific volume, pore size and uniformity, gas retention, and shelf life by addition of 2% RBPC [58].

For sensory evaluation and attributes, GF bread was determined by those attributes compared with the control bread as shown in **Figure 22**. High improvements in terms of appearance, color, smell, and overall liking were observed by 2% RBPC enrichment. In addition, the texture of GF breads (2% RBPC) was extremely accepted by panels, which is related to the firmness and relative elasticity values of 15.00 N and 54.11%, respectively. Taste was not significantly different between the breads containing egg albumin or RBPC. It was clearly seen that the addition of 2% RBPC displayed an important anti-staling effect in GF bread. The GF bread containing 1% RBPC and 1% egg albumin had an intermediate staling rate during storage, indicating a slower rate for development of crumb hardness than GF breads enriched with 2% egg albumin and the control recipe. Additionally, crumb porosity and sensory attributes were improved. RBPC also showed higher efficacy to inhibit bread staling than egg albumin. From this study suggested that RBPC could be used as a protein source as well as extending its shelf life for GF bread [58].

3.4.3.2 Gluten-free pasta production

Since manufacturers have long complained about gluten-free pasta for its apparent low cooking properties and reduced nutritional value, Phongthai et al. proposed to develop a multi-sourced protein-enriched gluten-free pasta [68] as depicted in **Figure 23**. RBPC is one of the alternative protein sources used in this study. Egg albumen (EB), whey protein concentrates (WP), and soy protein concentrates (SPC) were all enriched into rice flour-based gluten-free pasta in order to test their level of enhancement of cooking properties. In uncooked pasta, the RBPC-enriched pasta contained a soluble protein with a protein solubility of 22.9–27.59 mg/g sample when compared with that of WB 40.77–50.28 mg/g sample, EB (28.40–36.77 mg/g sample), and SP (16.56–21.18 mg/g sample), respectively. However, rice bran protein did not provide satisfied quality of gluten-free pasta. Obviously, RBPC influenced the highest solid loss, which was more than 7.5%. This may

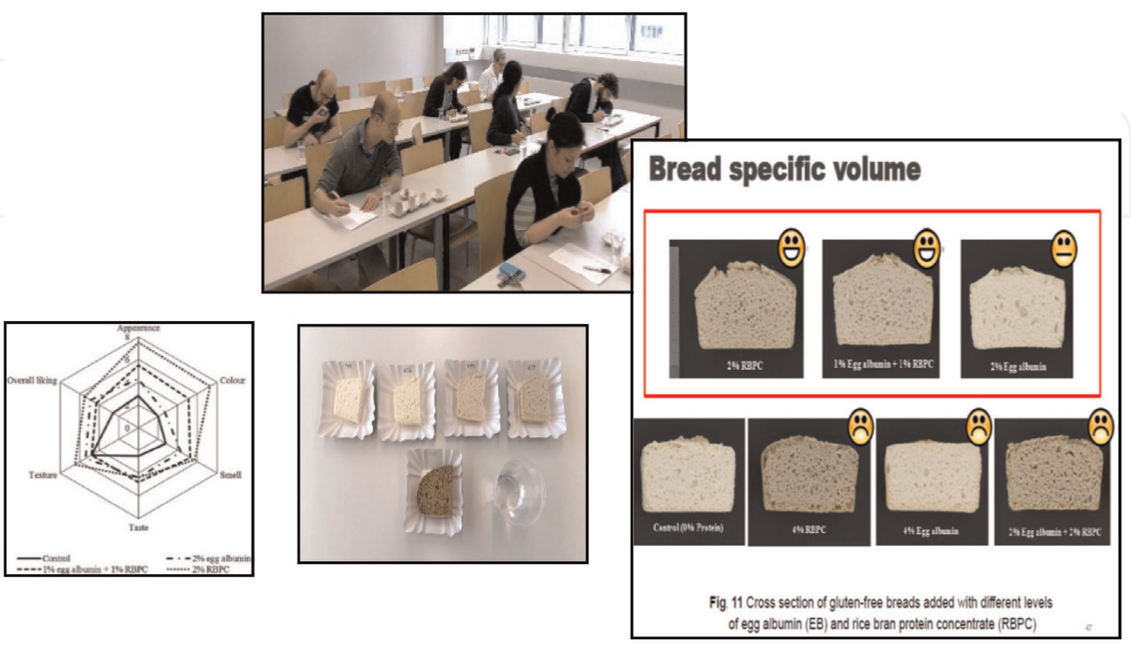


Figure 22.
Gluten-free breads enriched with different levels of egg albumin and RBPC and sensory evaluation attributes of GF breads. Source: Phongthai et al. [58].

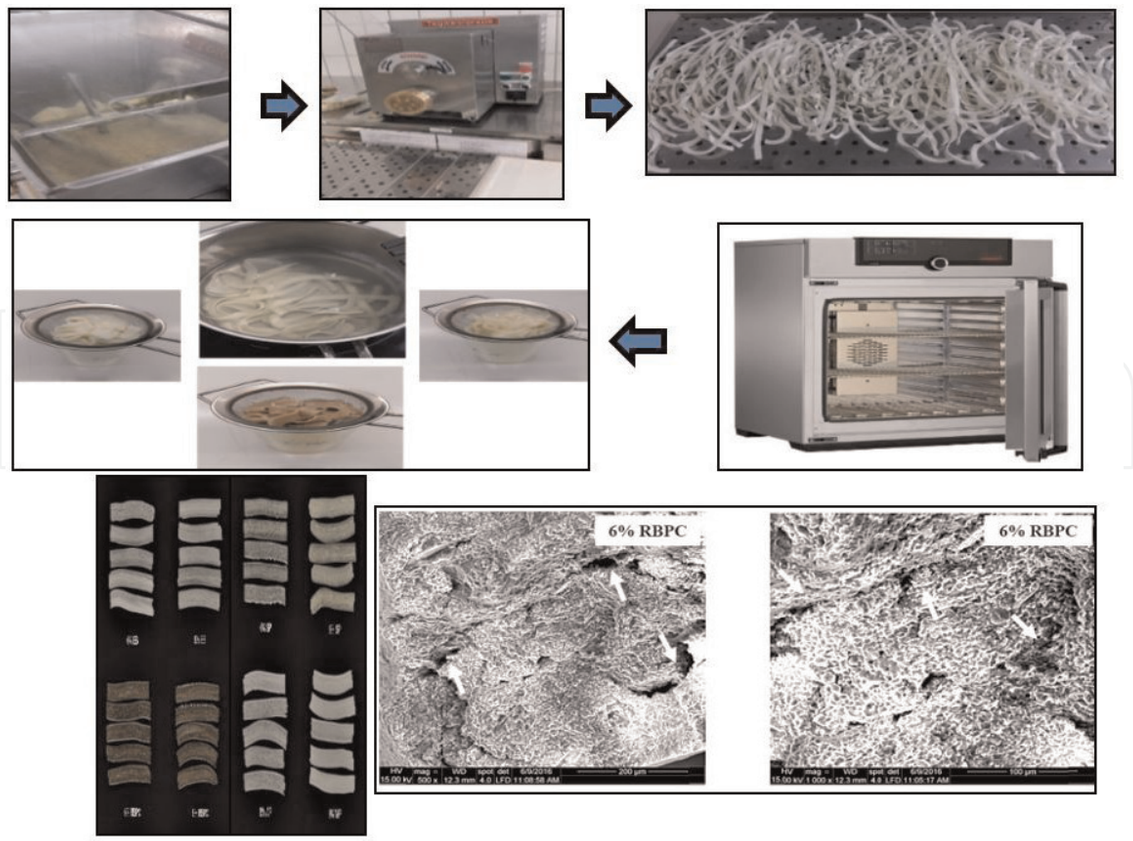


Figure 23.
Characteristic of uncooked gluten-free pasta and scanning electron microscopy images of rice flour-based gluten-free pasta enriched with 6 and 9% of egg albumen (EB), rice bran protein concentrates, soy protein concentrates, and whey protein concentrates. Source: Phongthai et al. [68].

be because RBPC is comprised of 6.91% fiber, which would be responsible for weakening the starch network, thereby increasing cooking loss. Besides, the addition of RBPC had a great impact on color parameters as shown in **Figure 23**, possibly because RBPC was obviously darker than the other protein sources. Egg albumen manifested the greatest potential for improving gluten-free pasta as it gave a short cooking time, a low cooking loss, and a firm texture.

3.4.3.3 Rice bran protein hydrolysates

Rice bran protein can be utilized by the production of rice bran protein hydrolysate (RBPH) by enzymatic techniques.The protein hydrolysates can be subjected to isolation and characterization for study possibility to be functional ingredient that benefits for human health. In the study of Phongthai et al. [64], RBPC that produced from alkaline extraction was used as raw material for rice bran protein hydrolysate production, and the hydrolysate was further fractionated by membrane ultrafiltration (UF, F1: molecular weight (MW) <3 kDa, F2: MW 3–5 kDa, and F3: MW 5–10 kDa) was used to fractionation of the hydrolysate [64]. Both RBPC and RBPH were digested by pepsin, and the combination of pepsin and trypsin under in vitro gastrointestinal digestion was investigated for its free amino acids (FAAs). The digestion affected the FAA content in each sample by RBPH had the highest FAAs content (34.44 mg/g) when compared with RBPC (22.40 mg/g) and pepsin-hydrolysates (27.07 mg/g). For antioxidant properties, it was found that the digestion by pepsin and pepsin-trypsin increased the DPPH radical scavenging activity of RBPC by about 3.1–4.9-fold. The digestion by pepsin and pepsin-trypsin could simulate the metal chelating efficacy of RBPC by about 2.17–2.21-fold. UF had a

positive effect with DPPH and ABTS but negative effect on the reducing activity of peptide fractions. However, the UF fractions and the RBPH with MW between 3 and 5 kDa showed outstanding activity to chelate. The majority of peptides with *m/z* at 1088 was supposed to be octapeptides, containing eight amino acids; a major peptide fragment in RBPH that has the highest antioxidant activity was detected at *m/z* 1088.

Rice bran protein hydrolysates at different degrees of hydrolysis (DH) (5.04, 10.37, and 15.04%) were obtained from RBPC from MAE and produced by Alcalase. The molecular weight (MW) of the rice bran protein concentrate and the PHs ranged between <11 and 100 kDa [62]. In addition, another RBPH was obtained from RBPC from UAE and further hydrolysis by Neutrase 0.8L and Subtilisin A [63]. The degree of hydrolysis for the rice bran protein by Subtilisin A, Actinase E, and Neutrase 0.8L was 20.03, 13.84, and 5.54%, respectively. The MWs of the isolated proteins ranged between <11 and 75 kDa. The RBPH exhibited greater scavenging activity on DPPH radical and ACE-inhibitory activity. The RBPH obtained by using Subtilisin A was efficient in reducing power and metal chelating activities. From these studies, it was found that that the partial hydrolyzed rice bran protein is more suitable for application in food systems rather than the non-hydrolyzed form [63].

Author details

Saroat Rawdkuen[1*] and Sunantha Ketnawa[2]

1 Unit of Innovative Food Packaging and Biomaterials, School of Agro-Industry, Mae Fah Luang University, Chiang Rai, Thailand

2 Postharvest and Food Engineering Laboratory, Graduate School of Horticulture, Chiba University, Chiba, Japan

*Address all correspondence to: saroat@mfu.ac.th

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