Application of Amphibian Skin and Skin Secretion

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Amphibians have been consumed as an alternative protein source all around the world due to their delicacy. The skin of edible amphibians, particularly frogs and giant salamanders, always goes to waste without further utilization. However, these wastes can be utilized to extract protein and bioactive peptides (BPs). Various BPs have been extracted and reported for numerous biological activities such as antioxidant, antimicrobial, anticancer, antidiabetic, etc. The main BPs identified were brevinins, bombesins, dermaseptins, esculentins, magainin, temporins, tigerinins, and salamandrins.

amphibian | frogs | skin | bioactive peptides | protein | alternative protein | collagen

1. Amphibians Production and Nutritional Composition

1.1. Production Status

Amphibian populations have been declining globally as a result of overexploitation paired with about more than 50% of amphibians listed as vulnerable, endangered, or critically endangered by the International Union for Conservation Nature (IUCN) Red List [1]. Although most known edible amphibians are frogs and salamanders, there is limited information regarding their global trade value. An integrated study using market surveys and interviews was conducted to study and discuss the domestic trade of frogs and salamanders. Most previous reports have been focused on frogs rather than salamanders. The overexploitation of salamanders (Chinese giant salamanders) for food has been reported, whereas their natural habitats have been found to be destroyed in the recent decade, particularly in China [2], and was categorized as a Critically Endangered species by the IUCN Red List of Threatened Species. Therefore, conservation has been initiated by collaboration between local government and farmers to fulfill the market demand and reduce overexploitation. For instance, the collaboration took place in Qinling region (China), where the government subsidized the initiation of the farm with an open investment from other locals [2]. On the other hand, frog farming and its culture have been part of Asian countries due to its low investment, relative ease of management, and its nutrition. The biotechnology trend has brought frog meat to popularity in the market, as proven by its increasing production mass [3]. For instance, in Thailand, slaughtered Asian bullfrogs have been exported to Hong Kong, Singapore, Malaysia, and Western countries with a capacity of at least 50 megatons per month. This amount has increased annually due to its high demand [4]. In southwest Nigeria, thirty-two (32) frog collectors were able to harvest a total of 2,738,610 frog species [5]. Frog meat is commonly exported as frozen deskinned legs where the other body parts are discarded without further utilization including the head, trunk, and skin [6][7].

Onadeko, Egonmwan, and Saliu [8] identified the predominant edible frog species in Nigeria as *Hildebrandtia ornate*, *Hoplobatracus occipitalis*, *Ptychadena pumilio*, and *Pyxicephalus edulis.* For non-consumptive utilization (such as medicinal and mythic purposes), *Bufo pentoni*, *Ptychadena mascareniensis*, *Tomoptera cryptotis*, and *Amietophyrynus* regularis have been used. The high annual demand in Nigeria has kept up with the large supply quantities where the excessive supply can be exported. In Indonesia, especially on Java Island, Crab-eating frog (*Fejervarya cancrivora*), Giant javan frog (*Limnonected macrodon*), and Grass frog (*F. limnocharis*), have been reported to contribute to 75.4%, 18.8%, and 5.8% of the total catch, respectively. Based on the Foreign Trade Statistical Bulletin by the Indonesian Statistical Bureau, a fluctuating trend of the export of frogs' legs was observed from 1969 to 2002 [6]. Gratwicke, Evans, Jenkins, Kusrini, Moore, Sevin, and Wildt [7] reported that Indonesia contributed around half of the global frog leg trade, accounting for USD 40 million where France, Belgium, and the USA were the predominant importers (75%). Unfortunately, no further update has been found since. Belgium, Luxembourg, France, the Netherlands, Singapore, and Hong Kong are the main export destinations. A production obstacle was also reported as during the dry season (February to August), it was sometimes hard to comply with the high demand of frogs' legs. Kusrini and Alford [6] projected a decline in the annual yield as it was affected by an increasing number of harvesters and middlemen as well as habitat and climate change, as more rice fields have been developed. Thus, it could lead to enlarge the gap between supply and demand. A decline in yield, along with a low export rate, might be explained by a higher rate of amphibian consumption among the locals, since they were familiar with the meat [9]. Therefore, by applying multiple coordinated approaches, their sustainability and productivity can be maintained without abandoning animal welfare, thus reducing the level of overexploitation [9][10][11].

1.2. Nutritional Composition

The most common edible amphibians are frogs and salamanders. Frogs' legs have been known as a unique culinary delicacy in Europe, the USA, Asia, Australia, and Africa [6][8]. It has been consumed as an alternative meat replacing chicken, since its taste is perceived to be similar to chicken. Most developed countries fulfill their frog meat demand by importing it from developing countries including Indonesia, Thailand, and Nigeria [1]. Meanwhile, salamanders are more popular in China than in other countries, particularly Chinese giant salamanders (CGS). The meat of CGS provide a tender texture with a similar taste to fish [12]. Both frogs and salamanders have been consumed due to their comparable nutritional value and eating quality compared to that of other land animals. **Table 1** presents the nutritional composition of edible amphibians from previous reports. The diversity of their nutritional values is influenced by different biodiversity, age, sex, feeding, farming, breeding type, and body part [13]. Fresh frog meats (fore-chest, thigh, and calf) consist of various moisture (68.9–83.4 g/100g), protein (10.5–21.2 g/100 g), fat (0.6–5.1 g/100 g), carbohydrate (0.5–4.6 g/100 g), and ash (0.6–5.0 g/100 g). CGS meat consists of moisture (79.0–82.3 g/100 g), protein (14.0–16.4 g/100 g), fat (2.5–3.5 g/100 g), and ash (0.7–1.1 g/100 g), however, their carbohydrate content has not been reported yet. The meat from frog species is comparable with that of CGS. He, Zhu, Zeng, Lin, Ji, Wang, Zhang, Lu, Zhao, Su, and Xing [12] reported that although different cultural environments and varieties of amphibians could yield different amounts, the main composition, nutritional efficacy, and medicinal characteristics were roughly the same. Here, the protein content of CGS was found to be slightly lower than frog meat. Nevertheless, both frogs and CGS meats were comparable with that of chicken and fish per edible portion. Some antinutritive compounds were found in *Pelophylax esculentus* and reported as dry basis matter including saponin (1.8 mg/100 g), tannin (5.4 mg/100 g), flavonoid (1.8 mg/100 g), and oxalate (2.8 mg/100 g) [14]. Thus, this could be a limiting factor in the consumption of edible amphibians.

Table 1. Nutritional composition of common species of edible amphibians.

Scientific Name (Common Name)	Body Part	Moisture (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	Carbohydrate (g/100 g)	Ash (g/100 g)	Reference
Frogs							
Bufo terrestris (Southern toads)	Whole body	68.9–74.8	16.8–17.5	2.8–5.1	nd.	3.2–3.8	[15]
Hoplobatrachus occipitalis (Crowned bullfrog)	Meat	71.7–77.8	16.9–19.5	1.1–1.8	4.6	5.0	[16]
Pelophylax esculentus (European frog)	Meat and bones	77.7	7.0	3.6	6.5	2.0	[14]
Pyxicephalus adspersus (Giant African bullfrog)	Thigh and calf	71.9–78.5	6.4–12.0	1.7–3.7	0.5–0.6	4.0–6.0	[17]
Rana catesbiana (American bullfrog)	Fore-chest	83.4	15.3	0.6	nd.	0.6	[18]
	Thigh	76.9	21.2	1.4	nd.	0.8	
	Calf	78.4	17.7	1.0	nd.	0.8	
Rana clamitans/Lithobates clamitans (Green frog/wood frog)	Whole body	76.2–78.8	15.3–16.7	1.5–3.2	nd.	2.7–3.3	[15]
Rana esculenta (Green frog)	Fore-chest	79.5	18.9	1.2	nd.	0.6	[19]
	Thigh and calf	79.7	19.2	0.7	nd.	0.6	
Rana nigromaculata (Black-spotted frog)	Skin	74.0	6.3	0.1	19.1	0.5	[20]
Rana ridibunda (Marsh frog)	Meat	79.8–82.8	10.5–15.7	0.7–1.5	0.9–1.3	nd.	[13]
Salamanders							
Andrias davidianus (Chinese giant alamander)	Meat	79.0–82.3	14.0–16.4	2.5–3.5	nd.	0.7–1.1	[12]

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Scientific Name (Common Name)	Body Part	Moisture (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	Carbohydrate (g/100 g)	Ash (g/100 g)	Reference
	Skin	67.6	29.1	1.21	nd.	0.5	[21]

nd.: not detected.

Amphibian skins are regarded as an agricultural by-product that is rich in proteins and lipids. As a collagenous source, it can be utilized to produce collagen, gelatin, and its hydrolysates. In general, gelatin and its hydrolysates have been utilized in food products as functional food additives or supplements. Focusing on their features, bioactive peptides (BPs) have been developed and formulated as food preservatives and as bioactive components of edible films and coatings [22]. Moreover, the application of collagen hydrolysate (CH) in the biodegradable film could effectively give more added value to the resulting CH in the food supply chain, considering the sustainability aspect. Protein hydrolysates containing BPs are used as components of biopolymer films not only to improve the mechanical properties of the film packaging, but also primarily as active ingredients extending the shelf-life of packaged foods [22]. The presence of BPs in protein hydrolysates makes it a functional ingredient that is applied in the food and medicine industries [23]. For instance, gelatin extracted from Asian bullfrog skin showed a similar gel strength to bovine gelatin [24][25]. Frog skin gelatin can be used as a biocompatible material, which is safe for human consumption based on cytotoxicity studies [24]. Gelatin from the skin of Chinese giant salamanders (CGS) exhibited good interfacial properties by enhancing foam expansion and foam stability. Thus, it has the potential to be used as a novel ingredient in food emulsion systems [21]. CHs have shown some bioactivities such as antioxidant and antimicrobial activities, enabling them to be utilized as a daily food supplement. Furthermore, the regular consumption of CH aims to enhance the production or regeneration of collagen in the human body [26]. Prior to the consumption of BPs, it is essential to consider the bioaccessibility of BPs during and after digestion, where the peptide modifications occurred (i.e., degradation and hydrolysis). This will generate smaller peptides with various sequences due to their bioactivities [27]. However, it was presumed that proline-containing peptides (such as CH) have a high resistance to hydrolysis by the digestive enzymes [28].

2.2. Application in Biomedical Industry

In the biomedical industry, amphibian skins and secretions have more potential for application. Nevertheless, collagen extracted from the skin of frogs and CGSs can be an alternative source of tissue engineering agents. Collagen can accelerate wound healing rapidly and effectively, which enhances immunomodulation [29][30]. This implies the functional property of collagen as a biomaterial scaffold. In addition, an in vivo study of alternative wound dressings using collagen, particularly from marine sources, showed a significant rapid wound healing mechanism in a rat model [26]. Nowadays, collagens have been utilized widely not only in the food industry, but also in the pharmaceutical industry due to their various bioactivities [31][32]. Another alternative source of collagen, especially from industrial by-products, would be potential and promising research in preparing wound dressings. The BPs of different frog skins possessed antimicrobial, anticancer, antidiabetic, immunomodulatory, and many more activities [33]. This also offers another application of BPs as a multifunctional and healthy supplement for particular consumer groups (i.e., elderly and /or patients with chronic disease). In addition, it suggests future cellular studies using AOPs in topical formulations for antiaging pharmaceuticals, or as putative neuroprotectors, which may assist in the development of therapeutic strategies aiming at controlling oxidative stress in neurological diseases [34]. BPs were evaluated on their anticancer activity, thus broadening their possibility as future cancer treatment agents. Nevertheless, BPs from amphibians can be formulated with other bioactive compounds to develop novel drugs or act as drug-delivery agents.

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