Nutritional and medicinal characteristics of Chinese giant salamander (*Andrias davidianus*) for applications in healthcare industry by artificial cultivation: A review

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Abstract

*Andrias davidianus*, i.e. Chinese giant salamander (CGS), is one of the largest and oldest amphibians existing in the world and is also one of the valuable biological resources of China. Wild CGS has been threatened with extinction in the past decades due to over capturing, deterioration of natural environment, the slow breeding and growth of the wild species in nature. However, in the past twenty years, with the breakthrough and progress of artificial breeding technology by artificial insemination, the number of artificially cultivated CGS has increased rapidly. Artificially cultivated CGS can either be released to the CGS living environment to increase the population in nature or legally applied in food and medicinal industry as a feedstock due to the unique nutritional and medicinal values of CGS as recorded historically. In this review, the nutritional components, bioactive components and medicinal activities of the artificially cultivated CGS will be summarized. The mucus, skin, meat and bone of CGS contain many different bioactive substances thereby having various medicinal activities including anti-aging, anti-fatigue, anti-tumor, therapy of burn and anti-infection and other physiological functions. This paper will further discuss the potential applications of the artificially cultivated CGS in healthcare industry and prospects of future technological development.

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Keywords: *Andrias davidianus*; Artificial breeding; Chinese giant salamander; Functional foods; Medicinal activity; Natural resource protection; Nutrition

1. Introduction

*Andrias davidianus*, also known as Chinese giant salamander (CGS), is one of the largest and oldest amphibians existing in the world and the only species of cryptobranchidae in China [1]. As shown in Fig. 1, CGS is the transitional species between aquatic and terriculous animal, and has lived on the earth for several hundred-million years. A CGS is about 50–150 cm in length and 2–20 kg in weight, and it breaths with gill in the young, while with lung in the adult [2]. CGS is lucifugous with small eyes and poor vision, so it is sluggish in action in the day. But in dark, it is active and get out of the caves for finding food which includes crab, fish, shrimp, aquatic insects and so on [3]. The CGS occurs in the regions around three major river systems (Yellow, Yangtze and Pearl rivers) in China [4]. It usually lives in the rivers at an altitude of 100–1200 m with clean water, fast-flowing tributaries, bare rubble and caves [5,6].

Wild CGS in nature is threatened with extinction due to the slow breeding and growth rate in nature and deterioration of habitat caused by over hunting and environmental pollution. For the conservation of the natural resources, wild CGS has been listed as a class II endangered and protected species of China and in the Appendix I of Convention on International Trade of Endangered Species (CITES, 2014). Because of lagged study...
and protection technology for wild CGS, it has also been listed in IUCN Red List data deficient species (IUCN, 2012) [8]. One of the traditional ways to protect the endangered wildlife is to establish nature reserves. To date, there are 21 natural reserves in China for CGS, covering most of the areas with record history of wild giant salamander. However, for lack of research and scientific management, many nature reserves fail to achieve their original goals for protecting wild CGS.

Another way to increase the population of wild CGS is through captive breeding, artificial cultivation and reintroduction into the nature [9]. Since 1960s, many domestic research institutions of China have been studying artificial breeding and cultivation technology for CGS. After 1990s, as the introduction of licensed use for aquatic wildlife through artificial cultivation, increase of private capital has been involved in the scientific research and artificial breeding of CGS in addition to the government investment. Up to 2015, the number of CGS breeding enterprises reached 2622 [10]. Over the past decade, the large-scale artificial breeding and cultivation technology has been successfully developed, and genetic study of the giant salamander has also made some progress [11,12]. Several CGS germplasm libraries have been constructed in the level of genomic DNA, cDNA, cell line and tissues [13,14]. By studying the genomic diversity, functional gene polymorphism and population behavior of the CGS species, the molecular behavior-driven analysis has been started, including classification, ecology, morphology, reproduction and origin of evolution [15–17]. The studies for the functional genomics and common viruses of CGS are also in progress [18–20]. Accordingly, improvement of the artificial breeding technology and the continued systematic biology study can help to restore the populations of CGS in nature. After nearly 20 years of the protection and management efforts for CGS, the illegal capture of the wild species in China has been banned completely. To increase the natural population of CGS, more than 60,000 CGS offspring generated by artificial proliferation and cultivation have been released to its natural habitats, and the number of CGS in nature had reached to more than 100,000. Meanwhile, according to the data of Ministry of Agriculture of China, the total number of farmed breeding CGS in China is about 12.49 million, and the number of seedlings amounted to 6.71 million until 2014 [21].

The capacity and increased number of the artificially cultivated CGS provide the potential resource not only for the protection and reproduction of the natural CGS population, but also for the large opportunity for food and medicinal industry by legally using the cultivated CGS as the feedstock from the viewpoint of the unique CGS nutritional and medicinal effects as described later. According to Chinese law, the wild CGS is a national second-class protected animal, but the second generation and after the second generation of the artificial domesticated CGS can be developed for utilization as food or other nutrition resources. The unique nutritional and medicinal characteristics of the artificially cultivated CGS have been gradually recognized, showing their potential application value in functional foods, cosmetics, and pharmaceuticals. This article summarizes the nutritional and medicinal values of CGS, as well as the development and utilization of artificially cultivated CGS for the applications in food and pharmaceutical industry.

2. Nutritional composition and values of CGS

2.1. General nutritional composition of CGS

CGS has been recorded historically to be a traditional food feedstock in China for thousands of years. The CGS meet can be made into delicious food with tender texture, delicious taste like fish, and high nutritional value known as “live ginseng in the water” [22]. Different culture environments and varieties of CGS can yield different contents of each nutrient, however, the main composition, nutritional efficacy and medicinal characteristics are roughly the same. As shown in Table 1, the water content of muscle of CGS was similar to that of grass carp and cultivated Acipenser sinensis, and higher than that of silver carp, Rana catesbiana and shark. The protein content of CGS was slightly lower than that of Rana catesbiana and shark, and was similar to that of grass carp, silver carp and cultured Acipenser sinensis. The fat content of CGS was slightly lower than that of silver carp and shark, and was higher than that of grass carp, Rana catesbiana and cultivated Acipenser sinensis. The ash content of CGS was slightly lower than that of other aquatic animals.

2.2. Composition of amino acids

Protein and amino acids contribute to the nutritive value and the flavor of food feedstock. The muscle of CGS consists of 18 amino acids. Among them, content of 6 flavor amino
Table 1
Contents of general nutritional composition in the meat of CGS and other common aquatic animals (%), dry weight [23–25].

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>Crude protein</th>
<th>Fat</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial breeding giant salamander</td>
<td>79.00–82.31</td>
<td>14.05–16.43</td>
<td>2.52–3.46</td>
<td>0.71–1.14</td>
</tr>
<tr>
<td>Grass carp</td>
<td>81.59</td>
<td>15.94</td>
<td>0.62</td>
<td>1.22</td>
</tr>
<tr>
<td>Silver carp</td>
<td>73.74</td>
<td>15.80</td>
<td>5.56</td>
<td>1.14</td>
</tr>
<tr>
<td>Rana catesbiana</td>
<td>76.31</td>
<td>18.26</td>
<td>2.10</td>
<td>1.10</td>
</tr>
<tr>
<td>Cultivated Acipenser sinensis</td>
<td>80.48</td>
<td>16.28</td>
<td>1.10</td>
<td>1.11</td>
</tr>
<tr>
<td>Shark</td>
<td>73.58</td>
<td>20.98</td>
<td>4.51</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Table 2
Composition and contents of fatty acids and their contents in the meat of CGS [29,31].

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Fat of CGS Muscle</th>
<th>Oil of CGS extracted with supercritical CO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>0.72</td>
<td>3%</td>
</tr>
<tr>
<td>C14:1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C16:0</td>
<td>21.74%</td>
<td>15.64%</td>
</tr>
<tr>
<td>C16:1</td>
<td>8.15%</td>
<td>16.4%</td>
</tr>
<tr>
<td>C16:2</td>
<td>0.68</td>
<td>–</td>
</tr>
<tr>
<td>C17:0</td>
<td>–</td>
<td>2.47%</td>
</tr>
<tr>
<td>C17:1</td>
<td>–</td>
<td>1.85%</td>
</tr>
<tr>
<td>C18:0</td>
<td>1.63%</td>
<td>2.87%</td>
</tr>
<tr>
<td>C18:1</td>
<td>37.52%</td>
<td>24.55%</td>
</tr>
<tr>
<td>C18:2</td>
<td>16.25%</td>
<td>8.28%</td>
</tr>
<tr>
<td>C18:3</td>
<td>3.12%</td>
<td>0.92%</td>
</tr>
<tr>
<td>C20:1</td>
<td>0.35</td>
<td>1.75%</td>
</tr>
<tr>
<td>C20:3</td>
<td>–</td>
<td>1%</td>
</tr>
<tr>
<td>C20:4</td>
<td>–</td>
<td>2.71%</td>
</tr>
<tr>
<td>C20:5</td>
<td>–</td>
<td>6.74%</td>
</tr>
<tr>
<td>C22:5</td>
<td>5.69%</td>
<td>2.64%</td>
</tr>
<tr>
<td>C22:6</td>
<td>–</td>
<td>4.27%</td>
</tr>
<tr>
<td>Saturated fatty acid</td>
<td>24.09%</td>
<td>23.98%</td>
</tr>
<tr>
<td>Unsaturated fatty acid</td>
<td>75.91%</td>
<td>76.58%</td>
</tr>
<tr>
<td>MUFA</td>
<td>46.69%</td>
<td>45.47%</td>
</tr>
<tr>
<td>PUFA</td>
<td>29.22%</td>
<td>31.11%</td>
</tr>
</tbody>
</table>

acids is accounted for 36.59–42.77% and 8 essential amino acids is accounted for 38.89–40.72% of the total amino acids, respectively [26,27]. The ratio of essential amino acids to non-essential amino acids in the CGS muscle was 68.68%, and the essential amino acid index (EAAI) of CGS muscle was 65.93 – 81.65, respectively [26,27]. The amino acid score (AAS, namely, amino acid content (mg/g) in a sample protein/corresponding essential amino acid content (mg/g) defined by FAO/WHO scoring standard model) and chemical score (CS, the amino acid content of sample protein/the amino acid content of eggs) of CGS muscle were 1.660 and 0.810, which indicated that the amino acid content of CGS muscle was 1.660 fold of the standard model selected by FAO/WHO and 0.810 fold of eggs, respectively [28]. The protein of CGS muscle is rich in sulfur-containing amino acid, and its limiting amino acids are tryptophan and leucine. In the skin, 17 kinds of amino acids were detected, up to 21.86% of the total amino acids of CGS. Among them, 7 kinds of the essential amino acids were 5.91–9.85% of the total amino acids, respectively, and leucine and valine were the limiting amino acids [29].

Table 2 Composition and contents of fatty acids and their contents in the meat of CGS [29,31].

2.3. Composition of fatty acids

Fat of CGS is mainly concentrated in the tail of 2/3 to the tip of the tail and significantly reduced in the tail of 2/3 to the caudal fin. Only very small amounts of subcutaneous fat exist in the caudal fin [30]. There are 13 kinds of fatty acids in the muscle of CGS. The composition of the fatty acids and their content in CGS are listed in Table 2. Among the total fatty acids, the content of unsaturated fatty acids in the muscle of CGS was 75.91%, of which 46.69% was monounsaturated fatty acid (MUFA) and 29.22% was polyunsaturated fatty acid (PUFA) [29]. Ratio of unsaturated fatty acid and saturated fatty acid was 3.15, higher than that of beef 0.9, pork 1.4, egg 1.6, shrimp 1.3, catfish 1.5, and grass carp 1.80, indicating that fat of CGS was rich in unsaturated fatty [24]. The PUFA in CGS are mainly /H9275-3 and /H9275-6 polyunsaturated fatty acids, and the content of /H9275-6 and /H9275-3 in the CGS muscle are 10.26% and 20.85%, respectively, which is conducive to human health [31].

2.4. Mineral elements and vitamins

As shown in Table 3, the muscle of CGS contains mineral elements of magnesium, zinc, iron, calcium, phosphorus, manganese and copper. Among them, phosphorus, zinc, manganese and copper are abundant and are a good source in food for the body to replenish essential mineral elements. Every hectogram of CGS cartilage and muscle can provide 34.3% and 26.3% zinc of recommended nutrient intake (RNI), respectively. A considerable content of selenium is also detected in the muscle of CGS, every hectogram of CGS cartilage and muscle can contribute to 80.6% and 76.2% selenium of RNI, respectively. Moreover, calcium is quite abundant in CGS cartilage, every hectogram of CGS cartilage capable of contributing to 61.6% calcium of RNI. Vitamins are abundantly detected in the liver of CGS, and the contents of VB, VB2, VA, VD and nicotinic acid are 0.8, 3.7, 11.34, 0.017 and 21.9 mg/kg in dry weight, respectively. Every hectogram of CGS liver can provide 141.8% Vitamin A and 34% Vitamin D of RNI [28].

3. Bioactive components of CGS

The muscle of CGS contains more than 70 kinds of natural bioactive substances, which can promote human physiological activity, improve the physiological metabolism, promote protein synthesis, regulate the body’s immune function and enhance the human resistance to diseases [34]. As shown in Fig. 2, the liver,
Table 3
Mineral substances of farmed breeding CGS [27,32,33].

<table>
<thead>
<tr>
<th>Positions(mg/kg)</th>
<th>Mg</th>
<th>Zn</th>
<th>Fe</th>
<th>Ca</th>
<th>P</th>
<th>K</th>
<th>Mn</th>
<th>Cu</th>
<th>Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>858.2</td>
<td>15.35</td>
<td>19.1</td>
<td>162.15</td>
<td>1020</td>
<td>–</td>
<td>2.35</td>
<td>0.8–3.73</td>
<td>0.381</td>
</tr>
<tr>
<td>Skin</td>
<td>360.6</td>
<td>15.93</td>
<td>67.6</td>
<td>170.3</td>
<td>730</td>
<td>101.2</td>
<td>0.14</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cartilage</td>
<td>–</td>
<td>53.15</td>
<td>5.76</td>
<td>4926.9</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.403</td>
</tr>
</tbody>
</table>

Fig. 2. Bioactive substances of artificially cultivated CGS.

mucus, skin, fat, cartilage and other organs of CGS are also rich in different bioactive substances such as collagen, glycoproteins, bioactive peptides, metallothionein, bombesin and so on.

3.1. Collagen

As a category of biological polymer material, collagen is a kind of structural protein of extracellular matrix (ECM) and consists of one or several triple-helical domains (collagen domains) composed of α-chains [34]. Collagen plays the important role of binding tissue in animal cells and is the main component of animal connective tissue. It is the most abundant and most distributed functional protein in mammals and related to the function of various tissues and organs, accounting for 25–30% of the total protein [35]. It is also closely related to the occurrence of many connective tissue collagen diseases. At present, there are 27 different types of identified collagen proteins, which are referred to type I, type II, type III collagen and the like in concordance with the order of discovery, wherein type I collagen is the most abundant that is widely present in the skin, bone, blood vessels and other connective tissue [34]. Due to the unique triple helix structure, collagen has received stable molecular structure, low immunogenicity and good biocompatibility. Collagen is a physiologically active protein and has various bioactivities [36]. Gu et al. [37] extracted collagen from farmed breeding CGS by using acid (0.5 mol/L acetic acid) or enzyme (pepsin) at low temperature (4 °C). The physicochemical properties of two different types of extracted and purified collagen, acid-soluble collagen (ASC) and pepsin-soluble collagen (PSC), were studied. The results showed that both ASC and PSC were type I collagen and contained two α-chains and a dimer β-chains. The amino acid contents of ASC and PSC were 144 and 173 residues/1000 residues, respectively. The highest content in ASC and PSC was glycine, followed by glutamic acid and proline, and the lowest content was cystine. Both of ASC and PSC contained amide II and amide III bands and a series of absorption peaks between the two amide bands, indicating that the triple helix structure of collagen in ASC and PSC was relatively intact.

The collagen was abundant in the skin of farmed breeding CGS with the content of (19.00 ± 2.66) g/100 g (wet weight), accounting for 62.89% of the total protein in the skin [29]. Li et al. [38] prepared collagen from CGS skin by pepsin and papain hydrolysis, and the analytical results obtained by ultraviolet spectroscopy and infrared spectroscopy showed that the skin collagen peptide of CGS had a triple helix structure of collagen. The results of SDS-PAGE showed that the molecular weight of collagen peptide was less than 55 kD. The UV absorption
showed that the strong absorption peak of collagen was about 230 nm and contained two different $\alpha$-chains ($\alpha_1$ and $\alpha_2$). Preliminary study confirmed that the extract obtained from skin of CGS belonged to type I collagen. Li et al. [39] found the physical and chemical indicators of CGS skin collagen peptide were higher than that of the light industry standards. It contains seven kinds of essential amino acids, and has good nitrogen solubility index, water absorption, water retention, wettability, oil absorption, emulsifying stability, foaming foam stability, indicating that it can be used directly in food processing.

3.2. Glycoproteins and active peptides

Amphibian skin is an important organ for its survive, and it carries many physiological functions including breathing, moisture regulation, temperature control, excretion, reproduction, resistance to microorganism infection and defense of natural enemies [40]. Amphibian skin can secrete mucus to keep the skin moist, and to maintain air and water permeability. Meanwhile, many functional peptides are also secreted. At present, over 1900 antimicrobial peptides have been discovered in the skin secretion of amphibians, as well as various anti-oxidant peptides, wound repair peptides, neurotoxin-like peptides, bradykinin, insulinotropic peptides [41,42].

CGS skin mucus mainly contains glycoprotein and active peptides with complex and diverse bioactive functions. Glycopeptides with more bioactivities can be further obtained by the protease hydrolysis of glycoproteins. Wang et al. [43] found that the molecular weight of antimicrobial peptide from CGS skin secretion was about 4.3 kD. Li et al. [44] obtained CGS glycoprotein with the molecular weight of smaller than 4 kD, and the sugar chain connected to serine or threonine by GalNAc and has $-$Galβ1-3GalNAcα-0-Ser/Thr structure. Wang et al. [45] obtained enzymatic product of CGS meat by TOF-MS and the molecular weight of enzyme hydrolysate was smaller than 2 kD, with the content of 2% sugar and 93% protein. Qu [46] used acidic proteinase from Aspergillus acidophilus to digest CGS skin mucus, and the glycopeptides obtained were characterized. The result showed that the sequence of polypeptide was: KAPILSDSSCKSC, KLQGTWSWGSCQAKNC and VVH-SLVQVTANKVMVRM. Tong et al. [47] studied the eutectic point and freezing concentration process of CGS glycopeptide solution, and the obtained eutectic point was $-3.6^\circ$C. The physiological function of the glycopeptide might be related to its glycosyl structure. Chen et al. [48] extracted polysaccharides and monosaccharide components from CGS skin mucus. The results showed that the monosaccharide components of the alkali extract were mainly Man, Glc UA, Gal UA, Glc N, Glc and Gal, but the monosaccharide compositions obtained by different extraction processes remains different.

3.3. Essential fatty acids

Essential fatty acids are fatty acids that are required for health but human and some of other animals cannot synthesize and have to intake from food [49]. One of the most important essential fatty acids is docosahexaenoic acid (DHA), which is usually found in fish oils, egg yolks and algal oil. DHA has physiological effects on lipoprotein, blood pressure, cardiac function, endothelial function, vascular reactivity and cardiac electrophysiology, as well as potent antiplatelet and anti-inflammatory effects [50]. There is high content of essential fatty acids in CGS, and content of DHA in liver and muscle of CGS was 392 mg/kg and 1,463 mg/kg, accounting for 4.91% and 5.69% of the total fatty acids, respectively. Content of another important essential fatty acid EPA, which had been shown to have physiological effect on coronary heart disease, hypertension, and inflammation, were 190 mg/kg and 647 mg/kg in the lipid tail and muscle of CGS, respectively. Every hectogram of CGS muscle can provide 66.5% DHA and 40.4% EPA of RNI for an adult [51]. The content of other 3 essential fatty acids- linoleic acid, linolenic acid and arachidonic acid were 799, 286, 446 mg/kg in the muscle of CGS, respectively [28].

3.4. Metallothionein

Metallothionein (MT) is a kind of metal-binding protein with low-molecular mass (2–7 kD), containing high cysteine content (20–30%), no histidine and aromatic amino acids, which is widely distributed in organisms [52,53]. The main functions of metallothionein in organisms include scavenging free radicals, preventing anti-ionizing radiation, detoxifying heavy metals, participating in the metabolism of mineral elements and organism’s stress response, enhancing the organism’s ability to adapt to adverse conditions, affecting DNA replication, transcription and protein synthesis, preventing cell apoptosis and so on [54,55].

MT can be induced by metals, cytokines, hormones, cytotoxic drugs, organic chemicals and stress [56]. The content of zinc in the muscle of CGS is abundant and is several times of freshwater and marine fishes. The microelement zinc can induce the formation of MT, and the MT, which is abundant in the liver of giant salamander, can enter the body and bind with toxic heavy metal such as cadmium, mercury, lead, etc., to play the role of detoxification [57]. Li et al. [58] induced MT by cadmium, and obtained two MT subtypes by twice separation and purification of the liver and intestinal materials of CGS. The apparent molecular weight of the two MT subtypes was about 12 kD. UV absorption peaks appeared at 250 nm. The yield of liver MT was 628 g MT/g fresh liver while the yield of intestine MT was 185 g MT/g fresh intestine.

3.5. Bombesin

It has been reported that in addition to mucopolysaccharide protein, fibrous material and a variety of hormones, bombesin is also present in the mucus of CGS. Bombesin has a strong antibacterial effect in other species such as Fire-bellied toad and Chinese forest frog, and can be used as a new source of antibiotics for research and development. However, there is no relevant experimental study on the identification and content determination of the CGS bombesin at present.
4. The medicinal values of CGS

As a kind of an edible animal with a long history of traditional Chinese medicine use in China, CGS has been documented by ancient herbal books of successive dynasties. The *Classic of Mountains and Seas*, literally translated as the *Shan-hai Ching*, is a Chinese classic compilation of mythic geography and myths thousands of years ago. It recorded a kind of fish like giant salamander with sweet and elegant taste and the effect of treating malaria and dementia [59]. Traditional Chinese medicine believes that CGS is delicious and suitable for everybody. Eating CGS has the effect of nourishing Qi (a professional noun in traditional Chinese medicine with the similar meaning as spirit) and blood, benefiting wisdom, and has the role of adjuvant therapy for the diseases such as neurasthenia, anemia, dysentery and malaria. The skin, muscle, mucus, bone and other organs of CGS can also be used as traditional Chinese medicine. The meat of CGS is not only well known for its nutritional and tonic effect, but also has curative effect with anemia, cholera, dysentery, chills and the like. The stomach of CGS can promote digestion, resolve food stagnation, and has been used in treating children’s functional dyspepsia since ancient times. The skin powder of CGS can be used with tung oil as specific medicine for treating burn and scald in folk prescription. Modern research has revealed that CGS is rich in different bioactivities, including antioxidant activity, anti-tumor, antibacterial, anti-aging, therapy of burn, anti-infection and anti-radiation and so on.

4.1. Antioxidant activity

The human body counteracts the harmful effects of free radicals and other oxidants every day, and it is very important to consume some antioxidants from food to keep health [60,61]. Many studies have indicated that active polypeptides with high antioxidant activity can be obtained from CGS muscle and mucus after protease digestion. The antioxidant ability of CGS mucus polypeptides is related to the reproductive age, the molecular weight of mucus polypeptides and enzymolysis conditions.

Qing et al. [62] obtained 90.28% yield of the polypeptide from the muscle of CGS by enzymatic hydrolysis. The produced polypeptides were separated into 5 molecular weight fractions by using ultrafiltration membranes with different molecular weight cutoffs. The effects of the different molecular weight fractions of CGS peptides on the scavenging ability of 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH•) and superoxide anion radical (O2−•) were studied. The results showed that the high antioxidant activity of CGS fraction could be separated by using ultrafiltration membrane with molecular weight cut off of 2 kD or 0.3 kD. Cao et al. [63] compared the difference of antioxidant activities between enzymatic products of CGS meat, beef, pork, chicken and fish under different processing conditions by neutral protease. The results showed that the enzymatic product of CGS muscle had highest activities of antioxidant activity, solubility, emulsifying ability, emulsifying stability and oil and water absorption capacity, compared with that of other animals. Jin et al. [64] studied in vitro antioxidant activity of active glycopeptides from the skin mucus of CGS. They found that the CGS glycopeptides prepared from the skin mucus had the effect of scavenging hydroxyl radicals and showed a significant dose-response relationship. The hydroxyl radical scavenging rate could reach 62% when the concentration of the active peptides extracted from the skin mucus was 5.78 μg/mL. Wang et al. [65] extracted oligosaccharide peptides from skin mucus of 3 years and 6 years of child-bearing CGS ant tested their respective antioxidant activity. The results showed that the scavenging free radical capacity of 6 years of child-bearing CGS is higher than that of 3 years of child-bearing CGS. Wang et al. [66] studied the hygroscopicity and antioxidant ability of moisturizing creams made by adding glycopeptides prepared from CGS. The results showed that the glycopeptides had the good effect of moisture retention, anti-UV, anti-oxidation and other characteristics.

4.2. Anti-aging effect

The aging of human body has significant relationship with the formation of free radicals. Because of the continuous contact between the human body and environment, free radicals are continuously produced in the body due to the breathing conditions (oxidation reaction), external pollution, radiation and other factors, which can damage human cells and deeply influence the formation of lipofuscin, mutation of mitochondrial DNA, induction of cell apoptosis and synthesis of protein [67].

Huang et al. [68] used *caenorhabditis elegans* to establish the senility model, and evaluated the anti-aging function of CGS crude extract through the determination of *C. elegans* life expectancy, progeny, worm size, and adaptive capabilities to acute heat stress and acute oxygen stress. The results showed that the crude extract of CGS with the concentration of 50 mg/L could prolong the life expectancy of *C. elegans* and had no effect on the reproductive ability. Cai et al. [69] demonstrated the anti-aging activities of CGS meat with α-galactose-induced aging model mice, and suggested that the mechanism might be related to the rising content of serum superoxide dismutase (SOD), telomerase activity and the decreasing content of MDA. Yang et al. [70] evaluated the anti-aging effect of CGS meat slurry in different proportions via the fruit fly model. The results showed that CGS meat significantly prolonged the life expectancy, improved the reproductive ability, flying ability and cold resistant capability of fruit fly, strengthened the in vivo activity of SOD and lowered the activity of lipofuscin, indicating that CGS meat had the positive effect on anti-aging of fruit fly.

4.3. Anti-fatigue

The term of muscle fatigue is used to express a transient decrease in the capacity of performing physical actions [71]. Muscle fatigue can be measured as a reduction in muscle force, a change in electromyographic activity or an exhaustion of contractile function. The improvement of exercise tolerance is the most direct parameter of increased anti-fatigue ability [72]. The change of the pole Climbing time and swimming time of mice can be used as the indicator of anti-fatigue effect of foods or
bioactive substances. Li et al. [44] obtained CGS oligosaccharide peptides with the molecular weight of smaller than 4 kD by using CGS mucus treated by compound proteinase. Burden swimming experiment of mice in water was selected, and hepatic glycogen and lactic acid formed in the model mice were measured as the biochemical indicators for examining the CGS effects. The results showed that oligosaccharide peptide of CGS could significantly prolong the burden swimming time of mice, lower the muscle lactate levels, and improve the content of hepatic glycogen, indicating the strong anti-fatigue effect of the CGS oligosaccharide peptide. Cao et al. [73] found that the CGS meat powder could prolong the swimming time of mice, decrease the range of blood lactic acid, and improve the hepatic glycogen reserves, thereby enhancing its anti-fatigue effect. Guo et al. [74] used nutrition fodder containing 12, 24 and 36 mg/7.5 g CGS meat to feed mice by free choice feeding with normal feed as control, the anti-fatigue experiment was studied by burden swimming experiment and pole climbing experiment of mice after 20 days under the same environmental conditions. It was found that the capabilities of swimming and pole climbing of mice were proportional to the content of CGS meat added in the fodder.

4.4. Anti-tumor

Xu et al. [75] studied growth inhibitory effect of human lung adenocarcinoma cell A549 by CGS mucus glycoproteins through in vitro MTT assay. CGS glycoproteins obtained by alkaline extraction (AE), DEAE-52 chromatography (DC) and Sephadex G-100 chromatography (SC) purification was added to the cell culture medium at 40 μg/mL, and the highest inhibition rate of A549 cell growth by AE, DC and SC glycoproteins was 88.43%, 91.34% and 92.32%, respectively. At the same concentration of CGS glycoprotein obtained by different separation methods mentioned above, the order of inhibitory effect on A549 cell growth was SC > DC > AE. The inhibitory effect of CGS mucus glycoprotein on A549 cell growth increased obviously with the increase of culture time, and the inhibition rate was time-dependent within a certain concentration range. Compared with positive control of paclitaxel, the difference of inhibitory effect on A549 cell growth by CGS mucus glycoprotein was significant (P < 0.01).

4.5. Therapy of burn and anti-infection

The medicines for therapy of burn the functions with antibacterial, diminishing inflammation and accelerating concrecence toward traumatic surface of the sufferers [76]. The CGS skin mixed with tung oil has been used as a folk prescription and showed good treatment effect since ancient times, but the mechanism of the treatment has not been studied.

Wan et al. [77] mixed the mucus, fat and skin of CGS, sesame oil and beeswax to prepare three ointments named “nisugao”, “niyougao”, and “nipigao”, respectively. The experimental mice model with the second-degree scald and frostbite was used to evaluate the effect of ointments described above in terms of wound healing time of the burns and frostbite, and Mebo burn ointment, a corresponding commercially available product, was
used as control. The results showed that the groups of “nisugao” and “niyougao” could obviously shorten the wound healing time of mice and promote the wound healing process in both burns and frostbite mice than the group of Mebo burn ointment.

Chen et al. [78] extracted CGS mucus, skin and meat powder with 80% methanol solution to check the bacteriostasis effect. Strong bacteriostasis effect of CGS skin extract on Pseudomonas aeruginosa, Staphylococcus aureus and Salmonella was discovered, while no bacteriostasis effect on Escherichia coli was observed. However, the bacteriostasis effect of CGS mucus, meat powder and oil described above was not significant. Wang et al. [79] separated and purified the antibacterial peptide from CGS skin mucus by acetate extraction, and evaluated the anti-microbial activity by inhibition circle method. Anti-microbial activities on Gram-negative bacteria, Gram-positive bacteria and fungi were found in the CGS skin mucus extract. Electrophoretic assay showed that the molecular weight of the polypeptide obtained above was about 4.3 kD, and had strong alkalinity. They further determined the anti-microbial effect of antibacterial peptides from CGS skin mucus on the wounds surface of mice infected with P. aeruginosa. The results showed that compared with control group, the wounds treated by the antibacterial peptide group healed faster, and the weight loss, and mortality of mice were lower than the other groups. The antibacterial peptide group had stronger anti-infective effect on the wounds of ICR (Institute of Cancer Research) mice infected with P. aeruginosa than the control group. Wang et al. [80] also separated a kind of peptide from CGS exuviated skin with the molecular weight of about 15 kD, which had inhibitory activity against E. coli, S. aureus, Mycobacterium phlei 607, Penicillium chrysogenum and Aspergillus niger.

4.6. Other bioactivities

Chen et al. [81] studied the antiplatelet aggregation and in vitro antibacterial activity of CGS oligosaccharide peptides by enzymolysis with trypsin, neutral protease and papain. The results showed that the enzymolytic products of CGS mucus by trypsin and papain had significant ability in antiplatelet aggregation. Zhao et al. [82] studied the clinical efficacy of CGS oligosaccharide peptide against skin photoaging. The results showed that CGS oligosaccharide peptides could reduce the facial pigmentation, impact of ultraviolet light on the skin and wrinkles, and hence had the capability of repairing skin and delaying skin aging. Qu [46] found that CGS glycoproteins (GSGPs) had UV absorption from 280 to 315 nm, and UV damage tests showed that the application of GSGPs on ear skin of mouse could avoid the damage of ultraviolet radiation. Li et al. [38] studied the characteristics and bioactivity of collagen peptide obtained from CGS skin and found the protective effect on ethanol-induced liver injury in mice.

5. Summary and prospective

In summary, farmed breeding CGS is rich in nutrients such as high-quality protein, ideal proportion of amino acids and fatty acids, and desired mineral elements and vitamins. There are also different bioactive substances such as collagen, active peptides, essential fatty acids, metallothionein and bombesin, including bioactivities such as antioxidant activity, anti-tumor, antibacterial, anti-aging, therapy of burn, anti-infection and anti-radiation and so on. Artificial breeding CGS is a precious resource for protection of natural population and possesses high nutritional values for promising applications in healthcare industry. It is an important strategy to improve the endangered situation of the wild population and realize the effective utilization of artificial breeding resource of CGS by rational combination of resource protection and industrial development. The development of artificial CGS breeding industry can provide the strong scientific support for release of CGS seedlings to nature. On the other hand, developing the processing technology, product chain and brand products from the artificially cultivated CGS is of importance for the needs of healthcare industry and development of the rural green economy.

However, the evolutionary process of CGS, molecular mechanisms and data-driven proofs for the various nutritional and medicinal values, and the breakthrough and progress of efficient artificial breeding technology of CGS and industrial application have not been well studied, which seriously hinder the development of artificially cultivated CGS —based healthcare industry. The main reasons are lack of the comprehensive science and technology research for CGS, the immaturity of processing technology for food and pharmaceutical industry and the insufficiency of science popularization. Fig. 3 summarizes the key technology development needed for CGS healthcare industry, including genome sequencing and germplasm reconstruction, evolution biology, breeding technology, high density and large scale cultivation technology, disease prevention and control, processing and bioactivity evaluation of the functional components, product chain design, product standards of the artificially cultivated CGS and the safety traceability for the whole life cycle from the cultivation to the end users.

Conflict of interests

The authors declare that there are no conflict of interests.

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