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Values-added utilization of protein and hydrolysates from animal processing by-product livers: A review

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ABSTRACT

Background: Livers, by-products from animal processing, serve as an excellent source of high-quality protein and bioactive enzymes. The need for animal protein resources encourages the search for and use of unconventional supplies. However, animal livers are commonly sold unprocessed at a low price since their unique properties and high-value utilization have not been fully explored. Fully use of animal livers provides an opportunity to utilize existing resources of food protein to the utmost and reduce the environmental pollution from the meat production when these raw materials not properly processed.

Scope and application: Efficient and environmentally-friendly extraction of liver protein and its hydrolysates, biological activities and potential applications are reviewed in this contribution. In addition, value-added utilization of liver protein and its hydrolysates are discussed. Furthermore, certain liver proteins, bioactive enzymatic hydrolysates and natural enzymes, as well as their potential utilization in agriculture, food, and medicine are evaluated.

Key finding and conclusions: Innovative technologies guarantee effective conversion of animal processing by-products into biologically active protein hydrolysates and procurement of high activity proteases. Animal liver protein and its hydrolysates have attracted much interest due to their broad applications in food, healthcare, animal feed and veterinary products. However, there is a need for intensive research on biological activity of liver protein and its hydrolysates in organism as well as on the regulation in animal by-products. Therefore, liver protein and its hydrolysates produced at industrial level would offer a value-added product for meat processing industry, and helps achieving resource sustainability.

1. Introduction

Consumption of animal-based food has been closely related to the nutritional needs of the population. The global output of livestock and poultry, and aquatic products was, respectively, 2365 and 1778 million metric tons (FAO, 2018). As a result, a large amount of animal by-products are generated, including feathers, fish scales, blood, bones, skin and viscera, among others (Dong et al., 2014; Lapena et al., 2018). These by-products contain an abundance of protein, fat, carbohydrate, minerals, vitamins as well as other nutrients (Moutinho et al., 2017). The basic components of animal livers are shown in Fig. 1. Animal livers are important by-products in food processing industry as they account for 2–3% of the body weight of animals (Chen, 2006).

In general, animal livers are rarely eaten except in the East and Southeast Asia. Traditionally, in these regions, livers are cooked in soup preparation and braised cuisine (Lynch et al., 2018). Liver paste products have also been commonly developed with pork, goose and chicken livers, and they are classified according to processed raw or precooked animal fat (e.g., spreadable liver paste) (Xiong, Han, Kang, Zhao, Xu, & Zhu, 2016). In addition, liver protein powder may be produced as a value added product (Zou et al., 2019). However, the purity of liver protein powder may not be high. In addition, the functional properties

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such as solubility, emulsification and foaming characteristics of liver protein have rarely been investigated (Liu et al., 2012). In Europe, besides cooked lamb livers, there are more processed products of porcine, chicken, duck and goose livers (Abu-Salem & Abou Arab, 2010; María et al., 2017). The utilization of livestock, poultry, and fish livers as functional food, biological agents and animal feed has been reported in the existing literature (Fang et al., 2020; Zou et al., 2017, 2018; López-Pedrouso et al., 2020). However, livestock by-products are scarcely used in most areas because their use for food is often restricted by regulation. For instance, materials obtained from by-products of ruminants can not be used as ruminant feed due to the risk of transmitting bovine spongiform encephalopathy (BSE) (Martínez-Alvarez et al., 2015).

Most of biochemical reactions that constitute the metabolism of organisms are catalyzed by active proteins, and the immune response of humans mainly needs proteins (Mayor et al., 2018). Therefore, the development of protein and its bioactive hydrolysates have gradually become an important strategy to seize the forefront of life science and technology (Xu et al., 2017). In plant food, proteins from soybean and oats are considered as being of high quality. Meanwhile, other plant proteins such as those from rice, flour, fruit, beans and vegetables are incomplete as their amino acid composition lacks some essential ones. Even the high-quality proteins in soybean and oats, with some anti-nutrients, their use is limited compared with animal proteins in this regard (Bohrer, 2017). The increasing world population and the demand of food with high nutritional values are expected to increase the need for more animal-derived protein by the year 2050 (Lynch et al., 2018). However, most animal livers are sold at a low price and used to produce animal feed due to lack of appropriate technology and processing equipment (Pilarczyk et al., 2020). Liver protein hydrolysates have excellent process characteristics (e.g., acid-base and heat resistance, good solubility and water retention, etc.) and nutraceutical value (Bhandari et al., 2020), however their use is limited in food or nutritional supplements due to lack of detailed research. Therefore, effective utilization and development of value-added products of animal livers as well as realization of their sustainable development is an important topic in the animal food industry. As shown in Fig. 2, this contribution provides a thorough review, summarizing the green extraction, biological activities and interesting applications of liver proteins and their hydrolysates.

2. Green extraction of proteins and their hydrolysates from animal livers

Protein is the most abundant component in animal liver after water (Gomes et al., 2017). Protein and biologically-active enzymes are naturally present in livers, while their food processing characteristics need to be clarified (Potter, 2014). Additionally, hydrolysates from liver protein are bioactive substances that can be used to improve human and animal health. Therefore, it is necessary to develop efficient and environmentally friendly technologies for their extraction in order to fully utilize them and their hydrolysates. In the extraction process of liver protein, the animal livers should first be defatted as animal livers are rich in fat. Additionally, enzymes from animal livers should be inactivated before enzymatic hydrolysis of other liver protein through enzymolysis catalyzed by added exogenous enzymes to obtain liver protein hydrolysates.

2.1. Aqueous extraction

The aqueous salt solution and buffer systems are effective in solubilizing proteins and are most frequently used for their extraction. The use of dilute salt solution promotes proteins dissolution from natural plants and animal source. Ions from the salt combined partially with protein prevent protein denaturation in buffer solution (e.g., 0.02–0.05 M phosphate buffer) extraction (Gianfrancesco et al., 2011). The solubility of most proteins increases with increasing temperatures. Therefore, higher temperatures are conducive to dissolution, and thus it shortens the extraction time required. Nevertheless, higher temperatures may denature and inactivate the protein. Therefore, extraction of

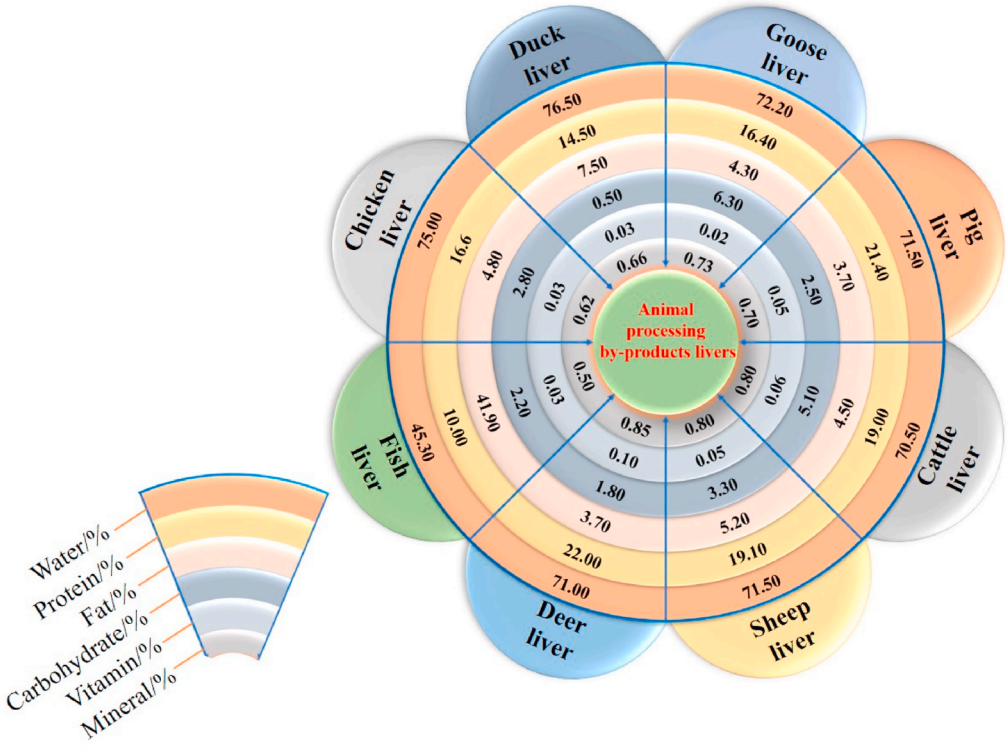

Fig. 1. Basic components (water, protein, fat, carbohydrate, vitamin and mineral) of liver from chicken, duck, goose, pig, cattle, sheep, deer and fish.

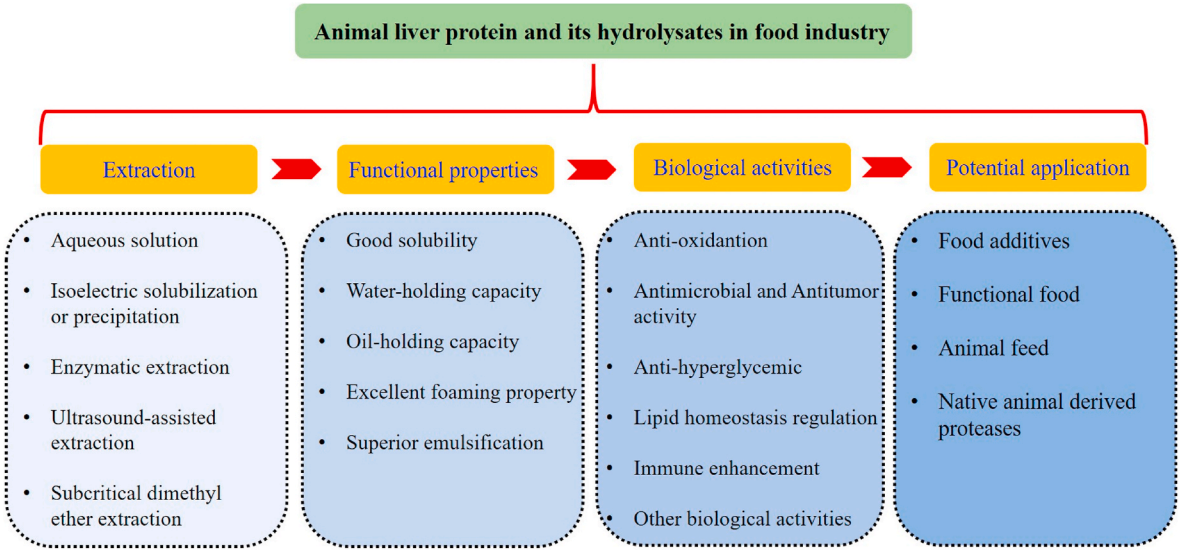

Fig. 2. Overview study of protein and its hydrolysates from animal livers.

protein and enzyme is generally carried out at a lower temperatures. Ferritin, a ubiquitous iron-binding protein, is abundant in animal livers (Friedman et al., 2011). As shown in Table 1, purified ferritin samples are obtained from liver by homogenization in dilute salt solution, centrifugation and salting out (Watanabe et al., 2011; Rao et al., 2018). Several liver proteins were obtained by using distilled water and phosphate buffer (Liu et al., 2012). Recently, aqueous extraction has also been reported as an interesting method for the preparation of enzymes and proteins from animal livers. Two trypsin isoforms (Klomklao & Benjakul, 2018), a novel copper zinc superoxide dismutase (Chafik et al., 2019) and water-soluble liver protein (Zou et al., 2019), were all isolated using phosphate buffer at different pH values, respectively. However, low extraction rate, large amount of solvent and complex extraction process of proteins are disadvantages that need to be overcome.

2.2. Isoelectric solubilization/precipitation extraction

Isoelectric solubilization/precipitation (ISP) adopts the principle that the lowest solubility of a protein is at its isoelectric point (IP), which is a safe and efficient extraction method for edible proteins (Zhu et al., 2010). For protein from different sources, the pH gradient of ISP varies, so the final yield and properties of protein are different when using these extraction conditions (Brouwer et al., 2019). For instance, yak liver protein can be extracted with 0.05 M NaOH solution and adjusted to IP to precipitate proteins (Liu et al., 2011). The components of chicken liver protein were extracted by acid/base solubilization (pH 2.0–3.5 and pH 10.5–12.0 to solubilize the majority of proteins, which were collected and recovered by IP (Xiong, Gao, Wang, Xu, & Zhou, 2016). Meanwhile, Zhao et al. (2020) and Xue et al. (2019) extracted goose liver proteins after treatment with acid (pH 2.0, 2.5 and 3.0) and alkaline (pH 11.0, 11.5 and 12.0) solutions. The use of acid-base reagents in ISP extraction leads to environmental concern, due to the use of chemicals, which is a disadvantage of using this method for industrial extraction of animal liver protein.

Table 1
Aqueous solution extraction of liver protein isolates from animal by-products.

Liver species	Biological proteins	Extraction conditions	Yield	Functional properties/ Biological activity	Reference
Chicken	Ferritin	Homogenization at 60 °C for 10 min and the obtained supernatant after centrifugation for acid treatment (pH 4.8) for 4 h	Not determined	Biotinylated hemin-binding activity Ferroxidase activity	Watanabe et al. (2011)
Japanese eel (*Anguilla japonica*)	A peptide derived from hemoglobin α	10% acetic acid stirred for 24 h at 4 °C and the supernatant collected by centrifugation	3.17 g/100 g w.w.	Antibacterial activity	Zhang et al. (2013)
Albacore tuna (*T. alalunga*)	Heat-activated alkaline proteinases	distilled water at a ratio of 1:9 (w/v) and the suspension was collected after centrifugation	Recovery of proteinases: 64.43 (Total protein, mg)	Not determined	Spipokar et al. (2015)
Tuna (*Thunnus alalunga*)	Two trypsin isoforms	50 mM Na-phosphate buffer, pH 7.0 containing 1 mM $CaCl_2$ at a ratio of 1:9 (w/v) and stirred continuously at 4 °C for 30 min and the suspension was collected	Trypsin A: 3.1 g/100 g w.w. Trypsin B: 19.2 g/100 g w.w.	High activity and stability over alkaline pH range and elevated salt concentrations	Klomklao and Benjakul (2018)
Sturgeon	Ferritin	The solid-liquid ratio of 1:3.8, pH of 8.12, and ammonium sulfate saturation of 57%	0.00329 g/100 g w.w.	Not determined	Rao et al. (2018)
Camel (*Camelus dromedarius*)	a novel copper, zinc superoxide dismutase	Potassium phosphate buffer (50 mM, pH 7.8, 1:2, w/v) was added and the supernatant was collected. By ethanol-chloroform treatment, the hemoglobin was removed. With salting out (K_2HPO_4, 300 g/L) effect and acetone precipitation	Ethanol-chloroform: 55.19 Salting out: 33.82 Acetone precipitation: 13.27 (g/100 g w.w.)	Antioxidant activity	Chafik et al. (2019)
Chicken	water-soluble liver protein	PBS (0.02 M, pH 7.2) at 30 °C and the suspension precipitated with saturated ammonium sulfate for 12 h	78.7 g/100 g w.w.	Emulsifying activity	Zou et al. (2019)

2.3. Enzymatic extraction

Enzymatic extraction (EE) breaks down the high-molecular-weight proteins into low-molecular-weight polypeptides and promotes protein release from raw materials (Tabtabaei & Diosady, 2013). The selection of enzymes is important for EE as the high enzyme activity can be only obtained under the optimum temperature and pH. For instance, chicken livers are usually hydrolyzed by using alcalase, neutrase, protamex, flavourzyme and papain at different temperatures and pH (Table 2). The results from these studies indicated that alcalase had the strongest hydrolyzing capacity and the hydrolyzates so obtained were with less bitterness, while flavourzyme hydrolyzates had the best sensory quality (Ju et al., 2010). Chou et al. (2014), Yang et al. (2014) and Chen et al. (2017) used pepsin to produce chicken liver protein hydrolysates (CLPHs), and the resultant products had excellent antioxidant, anti-inflammation, anti-fibrosis and cardiac protection activities. Furthermore, Xiong et al. (2020) employed trypsin (4000 U/g) to obtain CLPHs with desired degree of hydrolysis after enzyme denaturation. On the other hand, Shimizu et al. (2006) found that swine liver protein hydrolysates (SLPHs) reduced body fat in Otsuka Long-Evans Tokushima Fatty rats. Other SLPHs prepared by using trypsin enhanced the protective effect on liver injury, which was confirmed in a colon-targeted delivery system in a rat model study (Li et al., 2013). Enzymatic extraction degrades larger molecules of raw materials into smaller ones, which enhances hydrophilicity and hydrophilicity, and thus it increases the solubility of protein in the extraction solvent, and reduces the amount of solvent and production cost (Zou, Li, et al., 2017). At the same time, the bitterness of certain peptides is caused by hydrophobic amino acids, and carboxypeptidase is commonly used in enzymatic debittering (Yin et al., 2019). In other studies, alcalase, pepsin and papain were used to hydrolyze swine liver protein and the highest yield of SLPHs was 20.9% (Yu & Tan, 2017), and the highest antioxidant and antimicrobial activity of SLPHs was presented by trypsin (Verma et al., 2019b). Additionally, antioxidant peptides from SLPHs were obtained using alkaline phosphatase, bromelain, flavourzyme and papain with different pH and temperature conditions (López-Pedrouso et al., 2020). These investigations indicated significant value of animal livers for generating bioactive peptides.

2.4. Ultrasound-assisted extraction

Ultrasound-assisted extraction (UAE) is regarded as a “green technology” to extract animal and plant protein because of its advantages of high efficiency, energy saving and environmentally friendly approach (Zhang et al., 2017). The treatment time, intensity, and extraction temperature are important factors when using this method. Therefore, the key to improve the extraction rate and yield is to find the appropriate parameters for ultrasonic extraction. Response surface methodology was employed to optimize the extraction conditions of liver protein in UAE, and the yield of duck liver protein was 75.0% at ultrasonic power 265 W, ultrasonic time 42 min, NaOH concentration 0.80%, and solvent/raw material ratio 70 (Zou et al., 2018). In this regard, the influence of

Table 2
Enzyme extraction of liver protein isolates from animal by-products.

Liver species	Biological proteins	Extraction conditions	Yield	Functional properties/Biological activity	Reference
Chicken	Hydrolysis animal protein	Alcalase at 60 °C, pH 8.0, enzyme concentration of 1.25% for 2.5 h	85.32 g/100 g w.w.	Not determined	Ju, Chen and Ni et al. (2010)
Swine	Liver protein hydrolysates	Deionized water with 0.5% trypsin (w/w) at 55 °C of pH 7.5 for 6 h	Not determined	Reversing carbon tetrachloride-induced liver damage	Li et al. (2013)
Swine	Lver hydrolysate	Two kinds of porcine liver hydrolysates (LH-1 and LH-2)	Not determined	Antioxidant, angiotensin converting enzyme inhibiting and anti-hyperglycemic activity	Inoue, Hamasaki, Hidaka, Miura, Fukahori and Maruyama (2013) Inoue, Hidaka, Miura, Yamada, Fukahori and Maruyama (2013)
Swine	Liver protein hydrolysate	Proteinase at pH 7.0 at 45 °C for 4 h	Not determined	Reduces body fat	Shimizu et al. (2014)
Chicken	Liver hydrolysates	Pepsin (3000 U/mg) at a 1:400 (w/w) ratio at 37 °C for 2 h and the filtrate lyophilized to obtained hydrolysates powders after filtering (No. 1 filter paper, 55 mm)	16.17 g/100 g w.w.	Antioxidant activities, inhibitory lipase and bile-acid binding ability, anti-inflammation, anti-fibrosis and cardiac protection	Chou et al. (2014) Yang et al. (2014) Chen et al. (2017) Wu et al. (2019) Wu et al. (2020)
Swine	Liver hydrolysate	Liver hydrolysate is obtained via an enzymatic degradation of livers	Not determined	Recovery from physical fatigue and sickness behavior	Nakagawasai et al. (2013) Nakagawasai et al. (2015)
Swine	Liver hydrolysates	Alcalase (pH8, 50 °C), papain (pH 6.5, 37 °C), pepsin (pH3, 37 °C) and the homogenate digested using enzymes at 1% (w/w) for 3, 6, 12 h	20.9 g/100 g w.w.	Antioxidant properties	Yu et al. (2017)
Monkfish	Liver hydrolysate	Protamex (2.5%, w/w) at 50 °C	Not determined	Anti-oxidation and anti-fatigue	Xu et al. (2017)
Swine	Liver hydrolysates	Enzyme:substrate ratio kept constant (1:100) for 6 h for alcalase and papain and 4 h for trypsin	Not determined	Antioxidant and antimicrobial activity	Verma et al. (2019)
Swine	Liver hydrolysates	Alcalase/protein ratio of 1.5% (w/w), the protein/water ratio of 20% (w/w) and enzyme/protein ratio of 1.5%	Not determined	Not determined	
Swine	Liver hydrolysates	Papain at 37 °C and pH 6, bromelain at 40 °C and pH 6, Alcalase at 50 °C and pH 8, and Flavourzyme at 50 °C and pH 5.5 with an enzyme: substrate of 1:100 (w/w) for 7 h	Not determined	Antioxidant activity	Lopez-Pedrouso et al. (2020)
Chicken	Liver protein hydrolysates	Trypsin (4000 U/g) at pH 8.0 at 50 °C at a ratio of 1:6 (w/v)	Not determined	Antioxidant activity	Xiong et al. (2020)
Swine	Liver hydrolysates	Flavourzyme at 50 °C for 30 min with an enzyme to substrate ratio (1:100; w/w, pH 5.5)	Not determined	Antioxidant and antimicrobial activity	Borrajo et al. (2020)
Swine	Liver hydrolysates	Liver hydrolysate (LH) is obtained by an enzymatic degradation of livers	Not determined	Improvement depressive-like behavior	Nakagawasai et al. (2020)
Swine	Liver hydrolysates	LH is obtained by an enzymatic degradation of livers	Not determined	High digestibility	Soares et al. (2020)

ultrasound-assisted hydrolysis on liver protein and the antioxidant activity of its hydrolysates were investigated by Yu and Tan (2017). Ultrasound pre-treatment was found helpful in producing favorable antioxidant hydrolysates from porcine liver, which was low cost and with high efficiency and yield. Furthermore, ultrasound can change the physicochemical and functional properties of proteins because of its shear stress and cavitation effects (Zou, Li, et al., 2017). Ultrasound cavitation effectively disrupted the van der Waals forces, hydrogen bonding, and hydrophobic interactions between molecules, and thus it modified the particle size distribution and affected the secondary and tertiary structure proteins (Li et al., 2016). In industrial production, round container tanks with large diameters are used to create ultrasound blank area, but this leads to the attenuation and loss of ultrasound energy.

2.5. Other extraction methods

In addition to the aforementioned methods, other efficient extraction methods such as subcritical dimethyl ether extraction (SDEE) and sucrose density gradient centrifugation (SDGC) may be mentioned. SDEE could remove lipids and water simultaneously and conveniently so that the resultant protein content is relatively high (88.51%) (Fang et al., 2020). SDGC is used to form a continuous/discontinuous density gradient in the centrifuge tube, and then the sample suspension is placed on the top of the medium, so the proteins are stratified and separated by gravity or centrifugal force. Wang and Zhao (2009) isolated crude extract of rabbit liver protein by using SDGC twice. As shown in Table 3, most of the above methods have the shortcomings of low extraction rate, difficulty to control the extraction conditions, and incomplete-type liver protein. Appropriate extraction methods should be applied to liver protein and its hydrolysates for specific applications (Shaviklo et al., 2017).

3. Biological activity of protein hydrolysates from animal livers

3.1. Antioxidant activity

Reactive oxygen species (ROS) are produced in the process of oxidative phosphorylation to maintain life metabolism. Appropriate ROS participate in the metabolism of the organism in order to maintain their health (Mittler, 2017). However, excessive accumulation of ROS leads to oxidative stress, resulting in metabolic disorder and various diseases (Obregón et al., 2017). Recently, research and development of efficient and non-toxic edible antioxidant protein hydrolysates has become a hot topic (He et al., 2019). SLPHs exhibited an excellent antioxidant activity in various testing systems (Inoue, Hamasaki et al., 2013; Klomklao & Benjakul, 2018; López-Pedrouso et al., 2020). For instance, antioxidant activities were enhanced by supplying CLHs in male mice and male rats (Chen et al., 2017; Chou et al., 2014). Meanwhile, a high antioxidant activity protein hydrolysate prepared from goose liver was produced by using the method of Chou et al. (2014). The antioxidative goose liver hydrolysates has been proven to improve the texture quality of goose liver paste and enhance its functional properties (Zhao et al., 2020). The proximity of low-molecular-weight peptides and free radicals resulted in their increased antioxidant activity. At the same time, a close relation exists between antioxidant capacity and amino acid profiles in low-molecular-mass peptides. For example, histidine has excellent metal chelating ability. Aromatic amino acids such as tyrosine and tryptophan provide hydrogen, and cysteine plays an antioxidant role by electron donation (Qian et al., 2008). Therefore, these LPHs could be used as preservatives in food and pharmaceutical products.

Table 3
Comparison of different extraction methods of liver protein.

Extraction method	Advantage	Shortcoming
Aqueous solution extraction	Good protein stability and solubility, low cost, simple and practicable	Low extraction rate, large solvent consumption and complex extraction components
Isoelectric solubilization/ precipitation extraction	Simple process, easy operation, low cost and suitable for industrial production	Large amount of solvent, changes of protein properties, environmental pollution and strong corrosiveness
Enzymatic extraction	Environmental protection, high extraction rate, good solubility and production of bioactive peptides bioactive peptides	Sample pretreatment, reaction pH and temperature control, inactivation of enzyme, and difficulties in large-scale production
Ultrasound assisted extraction	Environmental protection, high extraction efficiency, wide adaptability of extraction, low extraction temperature and wide adaptability	Ultrasound attenuation, loss of energy, difficult to ensure the safety of ultrasonic devices and difficulty of on-line non-stop maintenance
Subcritical fluid extraction	High extraction efficiency, less solvent consumption, low temperature and none damage on the heat sensitive components in the material	Airtight and oxygen free environment, incomplete-type protein and consumption of organic reagents

3.2. Antimicrobial activity

Antimicrobial peptides are important part of innate immune system for organisms. These are a group of short-chain peptides that can resist the invasion of pathogenic microorganisms (Sibila et al., 2019). The mechanism of bacteriostatic efficacy caused by antimicrobial peptides is not only due to binding with specific receptors on cell membrane, but also non-specific interaction with membrane phospholipids (Lopez et al., 2014). Concurrently, the antimicrobial peptides also have a broad antibacterial spectrum. Therefore, antimicrobial peptides have low drug resistance and provide ideas for the research and development of new antibacterial drugs (Shen et al., 2018; Mi et al., 2017). Using different amounts of liver protein hydrolysates (LPH-1, LPH-2 and LPH-3) was found to decrease aerobic plate counts, yeast mold counts and coliforms with an increase in LPHs concentration (Verma et al., 2019a). For instance, PLHs (30 kDa) exhibited the highest antimicrobial capacity in inhibiting the growth of *Brochothrix thermosphata* (Borrajo et al., 2020). After reviewing a large number of studies, most antimicrobial peptides with a net positive charge were found to act on anionic bacterial cell membrane and play an antibacterial role (Lee et al., 2014). Additionally, the antibacterial activity is also related to the secondary structure formed by the peptide chain and the biological characteristics of the antimicrobial peptides (Lee et al., 2017). The main mechanisms of antimicrobial peptides are divided into membrane targets and intracellular targets. Most antimicrobial peptides have antibacterial effects by damaging cell membrane. On the other hand, some antimicrobial peptides specifically bind to intracellular targets to inhibit the synthesis of cell biomacromolecules (such as nucleic acid, protein, etc.) and play an antibacterial role. Moreover, antimicrobial peptides could enter into mitochondria and affect the activities of enzymes in mitochondria, which cause material metabolism disorder, and it eventually lead to cell death (Su et al., 2020).

3.3. Antitumour activity

With the increasing pressure of environmental pollution and working conditions, the rate of major diseases, such as tumour, is increasing. For instance, excessive cell proliferation is an important cause of tumourigenesis (Brown & Razzaque, 2018). Screening new antitumour drugs from natural foods has become a hot topic in recent years. Guo et al. (2013) found that yak liver protein (BGP) inhibited the proliferation and promoted the apoptosis of human HepG2 cells, showing significant

antitumour activity *in vitro*. As shown in Fig. 3A, antiapoptotic protein Bcl-2 binds to proapoptotic protein Bax and triggers a change in the conformation of Bax in livers. Therefore, Bax dimerizes inserts into the outer mitochondrial membrane to interact with transcription p53, which results in the release of cytochrome *c* from mitochondria. In addition, the collagen hydrolysate injection from suckling pig liver showed the highest inhibition rate on HEPA hepatoma of tumour-bearing mice (Zou et al., 2007). Wang et al. (2004) found that polypeptide A from puffer fish liver possessed antitumour activity *in vitro* and *in vivo*, but this was not related to the dose of polypeptide A and had no strong selectivity to human cancer cells or normal cells. The antitumour activity of LPHs was attributed to hydrophobic amino acids and amino acid sequences of short-chain peptides (Halim et al., 2018). Due to its low drug resistance, high efficiency, low toxicity and broad-spectrum activity, research on antitumour bioactive peptides has become popular globally. With the intensive study on the antitumour effect and its mechanism of animal liver protein and peptides, it is expected that these products may well be used as efficient antitumour drugs in clinical applications.

3.4. Anti-hyperglycaemic activity

Diabetes is a common chronic disease and its pathogenesis is complex and closely related to genetic and environmental factors (Åkerblom et al., 1997). The number of diabetic patients has been increasing globally. Diabetes and its complications are the main causes of disability, reduced quality of life, as well as the early death of the patients (Sinclair et al., 2017). In this sense, liver hydrolysates have the effect of lowering blood glucose in SHR/NDmcrcp (SHR-cp) rats that showed spontaneously occurring metabolic syndrome-like abnormalities (Inoue, Hidaka et al., 2013). As shown in Fig. 3B, Huang and Wu (2008) studied the active peptide (S-8300) from spotted bamboo shark liver to repair the damaged β-cells and promoted insulin secretion, which led to a reduced blood glucose and increased content of antioxidants such as superoxide dismutase (SOD) and oxygen free radicals to maintain the dynamic balance of blood glucose in mice. The mechanism of hypoglycaemic effect of S-8300 is related to the decreasing plasma lipid of alloxan diabetic mice and alleviating the harmful effect of free radicals. Compared with other drugs, peptides have higher activity, smaller dosage requirement and lower side effects (Shivanna & Nataraj, 2020).

3.5. Regulation of lipid homeostasis

Obesity is caused by genetic and environmental factors (Singh et al., 1996). The development of products with preventive effects, the control and treatment of obesity has become a focus of current research (Joyce et al., 2020). Consumption of SPLHs significantly inhibited hepatic activities of lipogenesis enzymes such as glucose-6-phosphate dehydrogenase and fatty acid synthase, thus it slightly increased fecal excretion of total fat (Shimizu et al., 2006). Therefore, SPLHs could significantly reduce the growth and the weight of fat pad including perirenal and epididymal adipose tissues in rats. In other cases, CLHs, rich in amino acids, may inhibit lipase activity and increase of bile acid binding activity for prevention of myocardial and renal damage caused by high-fat diet (HFD) (Yang et al., 2014). At the same time, CLHs reduced the levels of inflammatory and fibrotic cytokines in the myocardium of HFD fed mice as shown in Fig. 3C (Wu et al., 2020; Yang et al., 2014). On the other hand, liver hydrolysate (LH) can significantly increase the level of tyrosine hydroxylase induced by concanavalin A in mice, and increased the exercise activity, which proved that LH could restore and prevent the movement disease behavior (Nakagawasai et al., 2015). Bioactive peptides prevent or treat obesity by controlling the proliferation and differentiation of adipocytes. Compared with other weight loss control points, it is relatively safe, convenient and has less side effects by inhibiting lipase activity. Therefore, LHs could be a novel food ingredient for suppressing body fatto people with genetic obesity and high-fat dietary habit in a niche market.

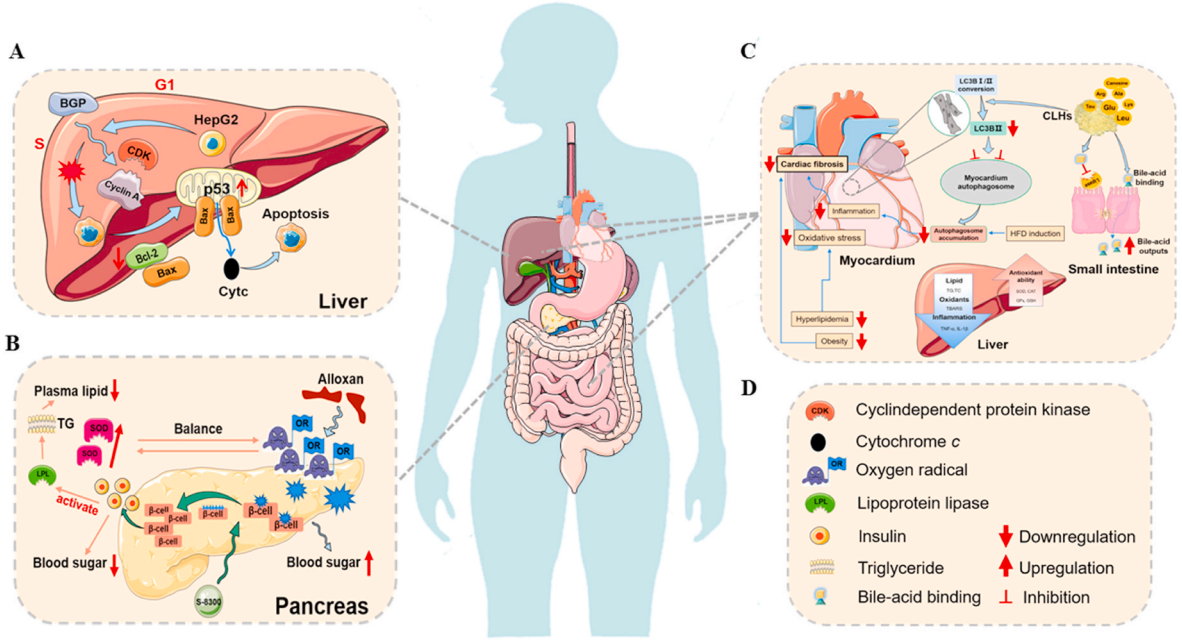

Fig. 3. Biological activities of animal liver protein and its hydrolysates. (A) Protective mechanisms of yak liver protein against proliferation and apoptosis of human hepatoma HepG2 cells. (B) Anti-hyperglycaemic mechanisms of peptide S-8300 in pancreas. (C) Protective mechanisms of chicken liver hydrolysates (CLHs) against high-fat dietary habit in three different organs. For small intestine, CLHs can inhibit lipase activity and improve bile-acid binding activity. For liver, bioactive compounds (amino acids and carnosine) exhibit a synergistic effect on lipid lowering and antioxidant status. For myocardium, high-fat diet habit causes the accumulation of autophagosome in myocardium, and CLHs can block autophagy pathway by lowering LC3II content. As a result, the reduction of autophagy accumulation can alleviate inflammation reaction and finally reduce cardiac fibrosis. (D) Icon illustration.

3.6. Immune enhancement effect

With new developments in cell biology and molecular biology, disorders of immune system and low immunity produce a variety of physiological diseases have been better understood (Chou et al., 2008). Moreover, these have a close relationship with the aging process and occurrence of senile diseases such as tumourigenesis, hypertension, diabetes and even psychosis (Sun et al., 2019). Fu et al. (2008) found that the newborn bovine liver peptides promoted the proliferation of splenic lymphocytes at various dosages. These peptides enhanced the delayed hypersensitivity, and increased hemolysin level in sera, the number of antibody-producing cells and the activity of NK cells. Protein hydrolysates from salmon by-products showed anti-inflammatory activity by restraining nitric oxide production and proinflammatory cytokines including tumour necrosis factor-α, interleukin-6 and -1β in RAW264.7 macrophage cells (Ahn et al., 2012). In another study, it was shown that one can synthesize or use microbial fermentation to express a variety of proteins or polypeptides as desired (Hemantha et al., 2012). However, the patented medicines report on the active ingredients that were first found in natural products or products synthesized or expressed (Liu & Feng, 2015). Therefore, it encourages the search for new immune active peptides from natural resources for drug use. Immunomodulatory peptides from animal sources are the most important part of immunoactive peptides.

3.7. Other biological activities

Apart from the above activities related to antioxidant, antimicrobial, and antitumour effects, liver hydrolysates (LHs) could protect liver injury and promote liver cell regeneration that has been proven to protect CCl_4-induced damage to the rats' livers (Li et al., 2013). Oral administration of LHs in rat resulted in a significantly lower blood pressure and anti-thrombosis (Ahn et al., 2010; Inoue, Hamasaki et al., 2013). Monkfish liver hydrolysates considerably alleviated fatigue in mice and may serve as a useful material to apply in functional foods (Xu et al., 2017). CLHs ameliorated inflammation and liver fibrogenesis by enhancement of alpha smooth muscle actin (α-SMA) gene and protein expression (Chen et al., 2017). LHs also present an anti-depressant effect on olfactory bulbectomized mice by increasing hippocampal phosphate-AMPK, phosphate-cyclic adenosine monophosphate response element-binding protein and brain-derived neurotrophic factor (Nakagawasai et al., 2020). The activities of liver protein hydrolysates are attributed to the active peptides and certain amino acids (taurine, aspartic acid, glycine, leucine, arginine, and alanine). Moreover, short chain peptides and free amino acids are easily absorbed in the small intestine or pass through the blood brain barrier. Therefore, animal liver is a good source of biopeptides that enhance the value-added utilization of animal processing by-products. Nonetheless, the promising bioactivities of liver protein have not yet been assessed in clinical trials.

4. Potential applications of animal liver protein and its hydrolysates

4.1. Food additives

Liver protein and LHs do not only contain essential amino acids that strengthen the nutritional function of food, but also control the texture and taste of food. Emulsifier and foaming agent are widely used in food processing. In this sense, the foaming and emulsifying functionality are precisely due to surface properties of proteins. Protein extracted from tuna liver (Fang et al., 2020) has high foaming and fat absorption capacity. In addition, Zouari et al. (2011) reported that turkey liver proteins show the highest foaming capacity at 5 or 10 g/L NaCl. With continuous improvement of people's concept of environment, economy, health and low carbon footprint, animal by-product proteins as foaming and emulsifying agents will be widely advocated. Zou et al. (2019) compared liver protein from traditional extraction and demonstrated that emulsifying activity and emulsion stability index of chicken liver protein by UAE were significantly improved. Therefore, liver protein with excellent foaming and emulsifying functionality can be used in the food processing industry in order to expand the application range of this byproduct (Fig. 4A).

The antioxidant and antimicrobial hydrolysates of porcine liver protein are also very important for food processing because they not only improve the nutrition and efficacy of food, but also extend the shelf life of products (López-Pedrouso et al., 2020). The Maillard reaction products from chicken liver protein hydrolysate and xylose had good degree of browning and antimicrobial capacity (Xiong et al., 2020) which could potentially replace some chemically synthesized preservatives and be utilized in food processing. For example, bovine liver hydrolysates with good antioxidant capacity were employed as food additives (Di Bernardini et al., 2011). LHs have broad market prospects and social benefits to improve the quality of life and health.

4.2. Functional food

The porcine liver protein hydrolysates (PLPHs) had excellent antioxidant and antibacterial activities as well as other bioactivities, and as such could be used for developing new healthy foods (López-Pedrouso et al., 2020). In recent years, antimicrobial peptides, as a rapid development product of human drug resistance, are also the focus of research due to their wide range of antimicrobial activities. The 30 kDa hydrolysate from porcine liver was found to have the strongest antibacterial activity against Gram-negative and Gram-positive bacteria, and its antibacterial rate was 116.7% at 10 h (Borrajo et al., 2020). Zhang et al. (2013) reported that a new antimicrobial peptide, AJHBα, isolated from the liver of *Anguilla japonica,* improved immunity and enhanced the body's disease resistance. Therefore, the antioxidant and antibacterial activities associated with these bioactive compounds could replace synthetic products currently used because of their adverse effects on human health.

Interestingly, monkfish liver hydrolysates significantly relieved fatigue in mice and showed an antioxidative effect in aging mice (Xu et al., 2017). CLHs alleviated renal lipid deposition and fibrosis, as well as cardiac fibrosis and inflammation in mice fed on a high-fat diet (Wu et al., 2020). In other studies, liver protein or its hydrolysates have been reported to inhibit tumour growth and regulate iron balance, or serve as immunomodulators and vaccine adjuvants and anti-depressants (Nakagawasai et al., 2020; Verma et al., 2019a). Therefore, LHs have attracted more attention in the development of agriculture, medicine, food and many other fields (Fig. 4B). However, studies on animal liver protein in functional foods are still limited, and there are only few reports on the structure and mechanism of its functional properties. These challenges and potential applications of liver protein could promote the sustainable development of animal processing by-products.

4.3. Animal feed

In general, animal protein is more complete than plant protein, so utilization of animal by-products prevents the loss of these potential high-quality protein resources. There are many studies on blood meal and feather meal, which are used in commercial aqua-feed (Martínez-Alvarez et al., 2015). However, protein and its hydrolysates from poultry viscera or porcine liver have attracted interest of feed producers (Fig. 4C). The reason ascribed to high-quality feed performance and animal growth is the presence of low-molecular-weight compounds with a high digestion rate. Shi (2008) found that animal liver proteins are better than fish meal in terms of nutrition and bioavailability. Liver protein powder is a kind of unconventional animal feed that can be developed and used as food attractant and feed nutrition additive in special cultures. For this reason, SLPHs were used for fish diets, and the productive performance of juvenile dourado *Salminus brasiliensis* was

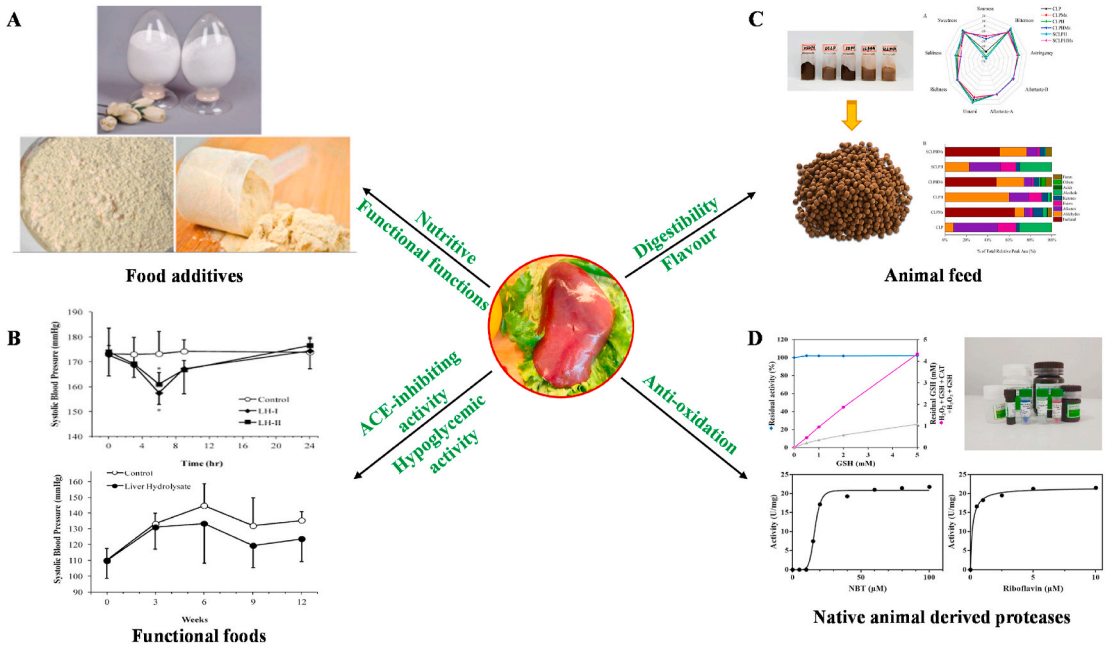

Fig. 4. Potential applications of animal liver protein and its hydrolysates. (A) Food additives. The liver protein powder or corresponding hydrolysates can be applied based on their excellent foaming and emulsifying properties. (B) Functional food. (C) Animal feed. The Maillard reaction products of liver manifest abundant volatile flavour substances according to the electronic tongue profile. (D) Native animal derived proteases. These proteases present good resistance against oxidative damage and efficient enzyme ability based on the descriptions provided by Chafik et al. (2017) and Chafik et al. (2019).

best achieved at 111 g kg^{-1} (Lorenz et al., 2018). Furthermore, protein hydrolysates extracted from poultry by-products and swine liver were used as protein substitutes for Pacific white shrimps (Soares et al., 2020). SLPHs were observed that 25% protein replacement of the control diet could increase the growth of Pacific white shrimps. Therefore, liver protein and LHs have attracted increasing attention, as an important sustainable animal feed material, especially in aquaculture.

4.4. Native animal proteases

The adaptation of organisms to the environment is inseparable from their specific material basis, and enzymes are very important functional substances closely related to the adaptability of biological environment (Fig. 4D). In this sense, catalase (CAT) is one of the key enzymes of biological defense system established in the process of evolution that widely exists in animals, plants and microorganisms (Ighodaro & Akinloye, 2018). Animal livers contain high levels of CAT, which reduces the hydrogen peroxide content and decreases its damage to biological cells (Dai et al., 2018). Chafik et al. (2017) reported that the yield and a specific activity of CAT from camel livers was 1.17% and 1132539.37 U/mg, respectively, which could provide the theoretical basis for living in the relatively severe environment, especially at high temperature. In a similar investigation, the relative enzyme activity of CAT from bovine liver reached 95.3% under optimal extraction condition, which is widely used in the dairy industry (Ma et al., 2010).

Trypsin from albacore tuna liver was optimally extracted using phosphate buffer (50 mM, pH 7.0) containing 0.2% (v/v) Brij 35 (Sripokar et al., 2016). The yields of two trypsins (A and B) isolated from the liver of albacore tuna were about 3.1 and 19.2%, respectively (Klomklao & Benjakul, 2018). In fact, Sripokar et al. (2019) demonstrated that trypsin from tuna liver was adopted to enzymatic hydrolysis for starry triggerfish muscle. The solubility of the hydrolysate was increased to 72.8% in a wide pH range. Trypsin is used as an adjuvant therapy for anti-inflammatory response and supplement intestinal digestive enzyme deficiency caused by pancreatic defect or congenital cystic fibrosis (Roxas, 2008).

Among natural tissues, animal liver tissues and blood contain the most abundance of superoxide dismutase (SOD). A new copper, zinc SOD (CuZnSOD) was obtained from camel (*Camelus dromedarius*) liver (Chafik et al., 2019). The higher optimum temperature, low activation energy and low optimum pH of this CuZnSOD may be related to the survival ability of camel in the severe desert environemnt. Öztürk-Ürek and Tarhan (2001) reported the isolation of CuZnSOD from chicken liver under the optimum pH 8.9 condition. This CuZnSOD had good pH stability in the pH range of 6.0–7.5 at 25 °C and presented good thermal stability up to 45 °C at pH 7.4. These results indicate that CAT, trypsin and CuZnSOD obtained from animal livers have potential application prospect in food, medicine and other fields. Due to the advantages of low price of animal livers, the research on the extraction technology of CAT, trypsin and CuZnSOD from animal livers has developed rapidly. The technology is simple and the extraction efficiency is high. The stability of these enzymes from liver extraction are excellent (Bougatef, 2009). At present, CAT, trypsin and CuZnSOD are also produced by microbial fermentation, but its disadvantages are that the production process is complex and the production conditions of these enzymes are strict (Wang et al., 2020). These natural enzymes from the liver have a wide range of functions, especially in helping to alleviate inflammatory reaction and improve cardiovascular and cerebrovascular metabolic regulation, making them a popular health care alternative to regular drug therapy.

5. Conclusion and future prospects

Animal livers, by-products of food processing, have been of interest to consumers for their rich nutritional and functional values. The market share of animal liver protein and its hydrolysate products in food, animal feed, healthcare and other industries, including natural health care products, is increasing year by year, with a good development momentum that has a high potential for expansion. However, the decrease in consumption of meat co-products in the recent years, has had a negative impact on the production cost, environment and greenhouse gas emission. Under increasing demand for high quality protein and environmental concerns, liver proteins from animal by-products must be fully utilized to address the needs of the market. The retention of

biological activity of liver protein hydrolysates in the human body is a problem that needs to be solved in diverse applications. Further, in order to develop the industry of liver protein and its hydrolysate products, it is necessary to pass relevant regulations for quality assurance aspects and safety issues to meet the needs of the international market. Therefore, with the improvement of processing technologies, legislation and potential safety hazard for animal livers, the preparation of animal liver protein and its hydrolysates is bound to become one of the most attractive emerging industries in future.

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