Potential Food and Pharmaceutical Application of Livestock Blood Proteins

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Abstract. Livestock blood generated in slaughterhouses is a huge impediment to the sustainability of the meat industry. Blood is produced in large quantities with high nutritional value but is underutilized, resulting in a giant waste of protein resources and severe environmental pollution. Many studies have been devoted to improving the added value of the slaughtered by-product blood, among which the biotransformation to obtain blood proteins or peptides is a noteworthy strategy. This article aims to evaluate the application potential of slaughtering by-product livestock blood in the food and pharmaceutical industries. Methods for the preparation of livestock blood proteins and peptides are summarized, and how livestock blood proteins and peptides can be used in the food industry as antioxidants, stabilizers, and biomarkers for meat product quality determination are also discussed. Moreover, functional components derived from livestock blood that can be used in the pharmaceutical industry are also presented. This article concludes by emphasizing the great potential of livestock blood being applied in food and pharmaceutical industries, which will hopefully minimize the environmental load of the meat industry by achieving the valorization of this underutilized slaughtering by-product.

Keywords: Livestock by-product; blood; blood protein; functional components; potential application.

1. Introduction

Human needs for nutrition are strongly correlated with the consumption of animal-based products, which contributed to a prosperous meat industry [1]. This resulted in the generation of abundant by-products such as bone, blood, viscera, and feathers amongst others [2]. Animal blood from the slaughterhouse ranks as the most problematic by-product derived from the meat industry, whose added value and market have been underappreciated [3]. According to the statistics from FAO, for the number of livestock slaughtered per year and the amount of blood per species, the blood production globally can be roughly estimated, as shown in Table 1. Livestock blood enriches a large amount of protein and functional components such as heme iron, superoxide dismutase, and immunoglobulins, which possess high nutritional value and beneficial functional properties [4]. Unfortunately, such an auspicious raw material is currently underutilized. Only a minor portion (about 30%) of the blood from slaughterhouses is processed for food [5], with most of the rest being disposed of as waste or utilized for some low-value products, such as agricultural fertilizers and livestock feed. Therefore, a higher added value of livestock blood and an intensive development of its novel applications in the food and the pharmaceutical industry are of great priority. A noteworthy high-value strategy is based on the enzymatic hydrolysis of livestock blood, which leads to blood proteins and blood-derived peptides that can serve as functional components [6, 7]. Typically, these high-value products are bioactive and promising in both the food and pharmaceutical industries.

This article aimed to discuss the potential applications of livestock blood proteins in the food and pharmaceutical industries beyond their role as ingredients in food. In this article, several different methods for the preparation of blood proteins will be described, including enzymatic hydrolysis and microbial fermentation, along with some emerging non-enzymatic assisted hydrolysis techniques. Afterward, the current research status and application potential of livestock blood proteins as antioxidants, stabilizers, and biomarkers in the food industry will be outlined. Finally, the potential applications of the functional components of livestock blood proteins in the pharmaceutical industry will also be analyzed.

Table. 1 Total amount of blood generated from several most-consumed livestock species in 2020

Livestock species	Number of slaughtered (head) *	Blood volume (L/head) **	Total amount of blood generated (L)
Chickens	70,767,577,000	0.15	10,615,136,550
Pigs	1,511,512,828	3.5	5,290,294,898
Cattle	293,196,735	17.6	5,160,262,536
Sheep	590,507,650	1.7	1,003,863,005

* Data accessed from FAO, available at https://www.fao.org/faostat/en/#data.

** Data accessed from Toldrà et al. [4]

2. Preparation of livestock blood proteins

Many studies have been conducted to prepare bioactive peptides from precursor proteins such as hemoglobin and plasma protein from animal blood. The most common methods of preparation include a) digestive enzyme hydrolysis, b) microbial fermentation, and c) proteolytic microbial enzymatic hydrolysis. Currently, there are also many non-enzymatic auxiliary means, such as subcritical water extraction, ultrasonic-assisted extraction, and macroporous adsorbent resin technique, which can achieve more efficient extraction of blood peptides [8].

2.1. Enzymatic hydrolysis

Hydrolysis using digestive enzymes is an effective approach to obtain bioactive peptides from food proteins. Gastrointestinal digestive enzymes that can be used include pepsin, trypsin, chymotrypsin, and peptidases at the surface of epithelial cells [9]. Apart from this type of enzymatic hydrolysis, peptides and amino acids can be released from proteins by some specific or non-specific proteases as well. These proteases are of broad origin covering almost all organisms of plants, animals, and microorganisms. Several of the most frequent proteases in industrial production include papain, bromelain, and alkaline phosphatase. As a conventional preparation method, enzymatic digestion has the advantages of mild reaction conditions, a controlled degree of hydrolysis, and high specificity [10]. However, this method has some defects, such as being time-consuming and having low hydrolysis efficiency.

2.2. Microbial fermentation

The microorganisms used for the microbial fermentation process, including some yeasts, fungi, and bacteria, generate protein hydrolases intending to hydrolyze proteins to obtain short peptides [11]. After choosing the suitable substrate and microorganisms, maintaining some external conditions such as temperature, acidity, and humidity are also necessary, allowing the product peptides to have better bioactivity [12]. Microbial fermentation offers certain benefits not available with other peptide production technologies due to the high diversity of microbial enzymes and the fact that a whole set of enzymes in the microorganism works compared to a single enzyme, which allows for the full release of precursor proteins with different sizes and sequences [11]. Also, this approach requires shorter production cycles and lower culture costs.

2.3. Non-enzymatic methods

Generally, bioactive peptides are extracted from blood proteins by enzymatic hydrolysis method, while techniques such as high-pressure, microwave, and ultra-sonic are often employed in the pretreatment step prior to the hydrolysis. These techniques are believed to facilitate parental protein to release bioactive peptides [8]. Especially ultrasonic-assisted enzymatic hydrolysis, whose main working principle for extracting proteins and peptides is the generation of bubble cavitation in biological matrices, is considered a non-destructive method suitable for mass production and applications to analyze whether the products are ideally degraded [9, 13].

3. Application of blood proteins in the food industry

3.1. As precursors of antioxidant and antimicrobial ingredients for food preservation

In recent years, food waste has become a global challenge. Food preservation technology has gained increasing attention. Typically, food preservatives are added to many foods to extend shelf life. These chemical preservatives are widely used because of their technological simplicity and relatively low cost. Nevertheless, they also suffer from criticism due to health hazards. Bioactive peptides with biocompatible and biodegradable properties are considered promising and safe alternatives to chemical preservatives, which are helpful in the preservation of various foods as antioxidant and antimicrobial ingredients [14].

Some antioxidant peptides such as EVGK, RCLQ, LDGP, and TGVGTK have been identified and isolated from duck plasma, which can serve as a major source of bioactive peptides that behave as bio-preservatives [15]. Furthermore, a study found that α137-141 peptide, the main component of hemoglobin, is a natural antimicrobial peptide that can reduce lipid oxidation by 60% at a concentration of 0.5% [16]. During the storage and sale of meat products, it can be a substitute for synthetic additives to prevent spoilage. The reports suggest that a sustainable technique, electrodialysis with ultrafiltration membrane (EDUF), can enrich the α137-141 antimicrobial peptide fraction from hydrolysates of different sources of by-products. The configuration of the electrodialysis cell is displayed in Fig. 1. This method has now been successfully applied to bovine hemoglobin and tested on ground beef, showing a 50% lipid oxidation reduction by the peptide recovered by the EDUF method [17].

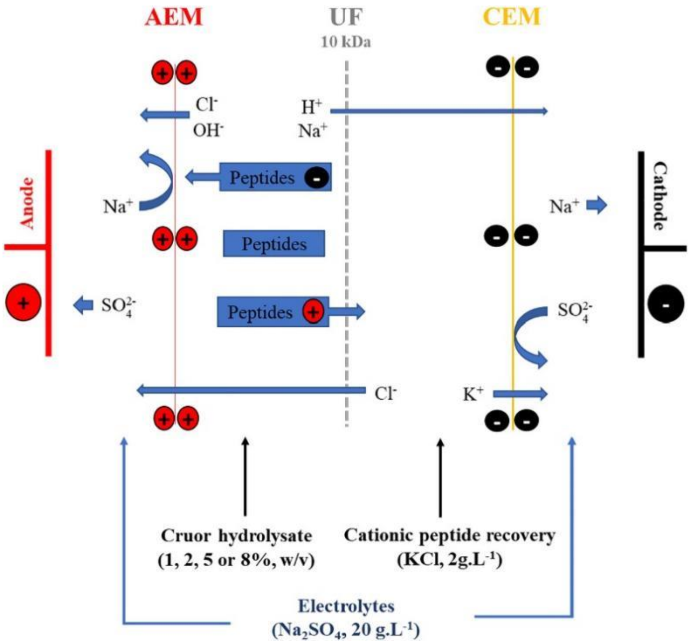

Fig. 1 Diagram of a cation configuration electrodialysis cell [17]

3.2. As a natural stabilizer for food emulsions

Food emulsions are non-homogeneous systems comprised of two immiscible liquids and a critical surface layer, which can be classified into two categories: oil-in-water emulsions and oil-in-water emulsions [18]. It is present in lots of everyday food items, including salad dressings and beverages, among others. However, such systems are highly unstable, and an emulsifier must be used prior to homogenization. One of the leading contributors to sensory and nutritional quality degradation of food emulsions is the oxidation of lipids. Low-molecular-weight synthetic surfactants, which are commonly used nowadays, have better interfacial diffusion properties than large biopolymers such as protein hydrolysates and polysaccharides but increase the possibility of lipid oxidation [19]. Under the combined consideration, protein hydrolysates, which are more biocompatible and sustainable, are considered a more suitable stabilizer, antioxidant, and adhesive.

Attention has been paid to the potential application of slaughtered blood proteins in food emulsions, and many studies have been successfully implemented on porcine plasma. Adding porcine plasma protein hydrolysate (PPPH) can reduce the formation of lipid oxidation products in emulsions [18]. It also increased the ζ-potentials and viscosities, which led to better emulsion stability. The researchers obtained O/W emulsions incorporating PPPH at different concentrations, stored for 10d at 22℃, as shown in Fig. 2. Also, porcine plasma has been demonstrated to provide an alternative to soybean isolate or sodium caseinate for more optimized stabilization in emulsified pork batter industrial mass production systems [20].

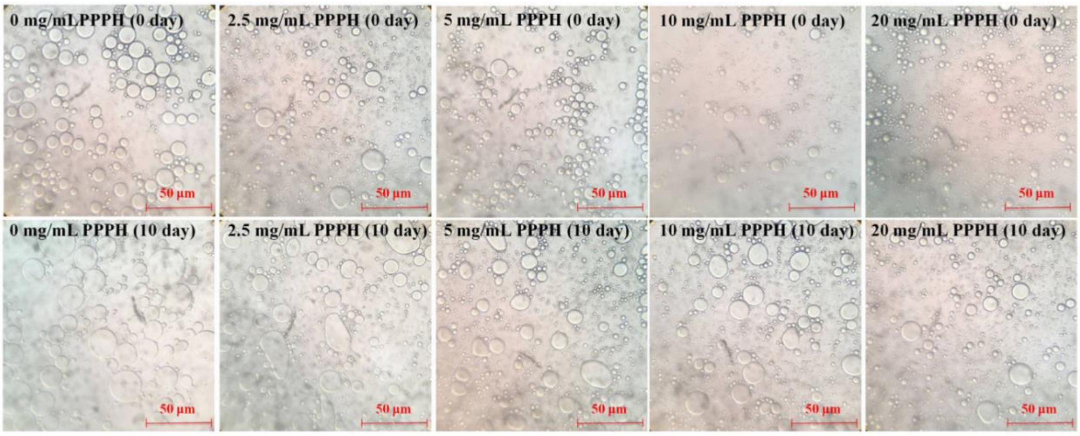

Fig. 2 O/W emulsion with PPPH added at 22℃ (day 0-10) [18]

3.3. As a biomarker for in vivo testing of meat product quality

Meat quality is a complex phenotype, and the methods currently commonly used to evaluate meat quality need to be performed after slaughtering. In recent years, metabolomics developments have significantly contributed to meat quality testing. Concentration fluctuations of some metabolites are a direct and sensitive reflection of the biochemical activity and status of muscle tissue, which allow the generation of several in vivo testing methods. It was found that some plasma proteins have the potential to assess meat product tenderness, and they can be used as low-invasive biomarkers to predict phenotypes prior to the slaughter of animals [21].

Body fluids such as livestock blood are considered preferable to muscle tissues for managing and testing commercial meat quality. Some proteins in poultry plasma have served the development of micro-invasive diagnostic tools that can be effective in predetermining poultry meat quality. A study discovered that chicken plasma biomarkers such as plasma 3-hydroxybutyric acid and raffinose could be promising candidate targets for the early onset of woody breast myopathy [22]. In addition, another study analyzed a series of plasma biomarkers, mainly amino acids and lactic acid, for the quality of duck and goose liver [23]. This would be an option for producers to determine the quality of duck and goose liver before cooking. 71 proteins that may be released in bovine plasma have also been examined, and 24 of them were found to be related to the tenderness quantitative trait loci by analysis. These plasma biomarkers have potential applications for the detection of beef tenderness [21].

4. Application of blood proteins in the pharmaceutical industry

4.1. As an iron supplement to treat anemia

Iron deficiency which causes anemia gradually becomes a global health issue. An estimated 1 billion people worldwide suffer from iron deficiency anemia [24]. The main causes of iron deficiency are inadequate intake of iron in the diet, impaired absorption of iron, excessive loss of iron, and a substantially increased demand for iron [25]. Currently many refined and processed foods are deficient in micronutrients, which leads to the failure to meet the body's iron requirements with diet alone. Furthermore, iron absorption in the body suffers from low efficiency and poor stability. Among the various strategies to control iron deficiency, developing novel dietary iron supplements using hemoglobin from animal blood is a promising direction.

Traditional oral iron supplementation products mostly involve inorganic iron, such as ferrous sulfate, or organic iron salts, such as ferrous citrate and ferrous gluconate. These free iron ions have many drawbacks, such as poor bioavailability, significant irritation of the gastrointestinal tract leading to adverse reactions, and the generation of endogenous free radicals [26]. Therefore, the food industry has shifted its attention to the chelating pathways of iron absorption and has devoted itself to developing peptide-chelated iron products. Hemoglobin peptide-chelated iron, obtained from livestock blood, is a biopeptide iron produced by chelating peptides generated from protein hydrolysis with ferrous ions. Compared with the ion absorption pathway, this chelating absorption pathway has the advantages of fast absorption, high absorption rate, good stability, and low toxicity [27]. Most current studies on peptide-chelated iron use animal blood such as pig and bovine blood as raw materials. An antioxidant peptide with 26.98% iron chelating activity has been isolated from porcine plasma protein [28]. Furthermore, it has been found that treatment of porcine blood powder with chymotrypsin and trypsin allowed a recovery of 70% heme iron in the form of soluble peptides, which showed an iron chelating activity of 16.36% [29].

4.2. As a source of functional components such as SOD, Ig and thrombin

Plasma therapy is both challenging and expensive in some low-income countries, while the abundance of livestock blood resources is drawing attention to its potential pharmaceutical value. Livestock blood contains many bioactive substances with medical and healthcare benefits, such as superoxide dismutase(SOD), immunoglobulins(Ig), thrombin, and prothrombin.

SOD is involved in the endogenous cellular defense system and performs antioxidant, anti-aging, and anti-tumor functions by scavenging excess free radicals in the body. It is approved for daily consumption and medical treatment because of its ability to cure gastrointestinal disorders and decrease diabetes and cardiovascular disease morbidity [30]. Immunoglobulin is a type of γ-globulin that plays a key role as a major antibody in the immune system and can effectively inhibit the pathogenicity of most bacteria and viruses. By orally administrated, Igs improve growth performance, regulate intestinal flora, and protect intestinal epithelial cells through the production of immunomodulatory mucins, which is more effective in regulating the immunity of the body [31]. Thrombin is the crucial enzyme in catalyzing the fibrinogens to convert to insoluble fibrin blood clots, and prothrombin is the precursor of thrombin [32]. Maintenance of normal hemostasis requires a defined system of pro- and anti-coagulant factors, and it is thrombin production that is essential for the functioning of this complex system [33]. It is thus clear that thrombin can be used as a highly effective hemostatic agent or adjunctive hemostatic drug for clinical treatment, mainly in medical, surgical, obstetrical and gynecological departments, and plays an important role in saving lives and treating bleeding disorders.

Some companies have already commercialized these functional components from animal blood. A bovine plasma serum concentrate comprising functional components such as Igs and transferrin, has been produced by a company in the United States [5]. And in the Netherlands, a product combined with thrombin and fibrinogen concentrate thrived from bovine plasma has already been put into the market [6].

5. Conclusion

This article presents the amount of livestock blood generated, utilization status, and current preparation techniques employed. The potential applications of livestock blood in the food and pharmaceutical industries are highlights in the discussion and evaluation. The preparation of livestock blood is mainly based on enzymatic hydrolysis and microbial fermentation, with many non-enzymatic assisted techniques such as ultrasonic-assisted methods and subcritical water hydrolysis. In the food industry, livestock blood can prevent food spoilage by preparing antioxidants, stabilize emulsions, and assess meat tenderness as a biomarker. A wealth of functional components such as heme iron, SOD, Ig, and thrombin can be extracted from livestock blood, showing excellent potential for application in the pharmaceutical industry. Harnessing these potentials, slaughtering by-product blood can contribute to the sustainability of the meat industry to a considerable extent.

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