



Invited Article

Blood-derived products for human consumption

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Introduction

Blood, the first by-product obtained after the slaughter of an animal, has long been used in European and Asian countries as an ingredient in traditional foods such as blood sausages, puddings, blood soups, breads and crackers (Mandal, Rao, Kowale, & Pal, 1999). For many years US slaughter houses used to discard blood as an unwanted by-product (Halliday, 1973) but its high nutritional value, coupled with serious disposal issues, has fueled recent research and industrial efforts to incorporate blood proteins into a wide range of food products. Commercial blood products, either from plasma or the cellular fraction of blood including whole blood, serve particular functions in different products. Although they are mainly used in meat products, primarily to increase protein levels and enhance water binding and emulsifying capacity (Mandal, et al., 1999), advances in food technology mean that blood derived products are beginning to be found as ingredients in non-meat processed food and dietary supplements. Consumers are often unaware that some of these products are now being used in sectors of the food industry that hitherto did not use blood ingredients, with these often being declared on the label merely by their brand name or the name of the protein. This is a serious issue for Jews and Muslims, who are forbidden to eat anything derived from blood as a result of the dietary restrictions imposed by their religions, as well as others who avoid blood-tainted food or any product of animal origin due to ethical, cultural, or health reasons, or simple personal preference. Regulatory guidance in the proper labeling of these products is now crucial to protect consumers and the development of effective analytical methods for the detection of hidden ingredients derived from blood in food products is thus urgently needed.

Usage of animal blood

Blood, which constitutes 3-5% of the live weight of an animal (Halliday, 1973) has traditionally been widely used in food preparations for human consumption in many societies. In Europe it is used to make blood sausages, biscuits, bread and blood pudding, while in Asia it is a vital component of food products such as blood curd, blood cake and blood pudding. These blood products, however, are not commonly consumed in the United States, where for many years blood was discarded as an unwanted by-product by

slaughterhouses. Given its significant solids content (18%) and high chemical oxygen demand (COD) (500,000mg O₂/L), the environmental problems caused by the disposal of the large quantities of blood produced are enormous (Del Hoyo, Moure, Rendueles, & Diaz, 2007). Previous methods of disposing of untreated blood, either by spraying it onto agricultural lands or dumping it into sewage systems, are now illegal due to the serious environmental pollution that results (Nowak & von Mueffling, 2006). Circumventing the immense cost inherent in disposing of slaughterhouse blood in an environmentally friendly manner has led to the development of ways to convert animal blood into forms that are

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useful as ingredients in both human food and animal feed.

The unique nutritional and functional qualities of blood and its derived products have also contributed to their increasing usage as food ingredients. Typically, bovine blood consists of 80.9% water, 17.3% protein, 0.23% lipid, 0.07% carbohydrate, and 0.62% minerals (Duarte, Carvalho Simoes, & Sgarbieri, 1999). Not only is it a high protein by-product, but its proteins have desirable functional properties, including binding, foaming and emulsifying capabilities. The bulk of the slaughterhouse blood produced is now utilized by the food industry, mainly as a gelling agent and as a natural colorant. Hemoglobin, which is present in the red blood cells, accounts for more than 50% of the total proteins present (X.Q. Liu, Yonekura, Tsutsumi, & Yoh Sano, 1996). The high iron content of blood, coupled with the high absorption of heme iron compared to non-heme iron, is particularly useful for food based strategies designed to combat iron deficiency anemia (IDA), a major global malnutrition problem (Kikafunda & Sserumaga, 2005; Walter, et al., 1993). Hence, efforts to fully utilize blood through the recovery of its proteins are both necessary and justified not only because of the environmental benefits that ensue, but also its potential nutritional, functional and economical benefits. The food industry currently uses about 30% of the slaughterhouse blood produced (Gatnau, Polo, & Robert, 2001). The United States Meat Inspection Act approves the use of blood in food provided that it is obtained by bleeding an animal that has been inspected and passed for human consumption as meat (9CFR 310.20). The remainder of the blood produced is utilized by the pet food, livestock, agriculture, pharmaceutical, medical, diagnostic and paper industries.

Blood and blood products used in food

Because of the many benefits that are derived from utilizing blood products as food ingredients, a number of such products are available on the market to serve specific functions. Blood is made up of two fractions, namely the cellular fraction comprising the red blood cells, white blood cells and platelets and the plasma fraction, with the

former suspended in the latter. Blood and blood products are obtained mostly from bovine and porcine sources, as the use of blood from other species is generally not practicable (Karasz, Andersen, & Pollman, 1976). These products are either derived from whole blood, the plasma fraction, the cellular fraction or from proteins isolated either from the plasma or cellular fraction.

Plasma and derived products

Plasma contains about 7.9% protein, consisting principally of immunoglobulins (4.2%), albumins (3.3%) and fibrinogen (0.4%) (Howell & Lawrie, 1983). Of the two blood fractions (plasma and cellular), plasma and the products derived therefrom are more widely used in the food industry because of their neutral taste and lack of the dark color typical of the cellular fraction. In the meat industry, however, the predominant role of plasma products is as a binder because of their ability to form gels upon heating. For example, the plasma proteins fibrinogen and thrombin are selectively cryo-precipitated from plasma and used as a natural binder in whole muscle processing. This product, patented by the Dutch company Harimex B.V., is sold commercially under the brand name Fibrimex® (Wanasundara, Pegg, & Shand, 2003). This restructuring process reduces waste by allowing the entire muscle as well as the trimmings (which would otherwise be used in the production of cheaper comminuted meat products) to be fully utilized (Flores, Boyle, & Kastner, 2007). Also, because Fibrimex® functions as a cold-set binder it can be applied to both chilled and raw meat products without compromising the product quality. Plasma and its derived products hold a special place in the food industry, as in addition to their use as binders plasma proteins are good emulsifiers and are thus frequently used in emulsified meat systems, where they serve as an abundant source of nutritionally beneficial protein (Mittal, 2005). They are also used as protein supplements and fat replacers in meat products such as sausages. Major muscle proteins like myosin have the ability to cross-link with plasma proteins, enhancing resistance to endogenous protease degradation. Thus, dried plasma is used as an inhibitor for endogenous proteases in surimi-

type products made from certain species of fish in order to inhibit degradation by endogenous proteases (Viana, Silva, Delvivo, Bizzotto, & Silvestre, 2005; Wanasundara, et al., 2003).

Though plasma products are used mainly in the meat industry, they also find applications in other sectors of the food industry. For example, spray-dried plasma can be used as an egg substitute in bakery products because of the foaming and leavening properties of blood plasma proteins (Ockerman & Hansen, 2000; Raeker & Johnson, 1995). Substituting spray-dried plasma for eggs only produces cakes with desirable qualities if the substitution is partial, but as egg products are among the more costly ingredients used in the bakery industry even partial substitution can reduce the product cost substantially (Raeker & Johnson, 1995). Pasta is another food that is widely consumed in many parts of the world. It is typically produced from durum wheat, which is both expensive and in short supply, necessitating its replacement with lower grade flour. Unfortunately this tends to be protein-deficient, so incorporating spray-dried blood plasma into biscuit flour, which is widely available and much cheaper, allows manufacturers to produce protein-rich pasta (Yousif, Cranston, & Deeth, 2003). A plasma product produced by Proliant Inc., IA, and sold under the brand name Immunolin® is also used as an active ingredient in dietary supplements to boost immune function (www.proliantinc.com/health/products/sports-nutrition.asp).

RBCs and derived products

Separation of blood into its component fraction yields 52-70% plasma and 30-48% of RBCs (Ockerman & Hansen, 2000). However, the use of hemoglobin-containing blood products such as whole blood, red blood cells or hemoglobin as food ingredients has not been as popular as those products derived from plasma. This is primarily as a result of the heme component of hemoglobin, the major protein in RBCs (and whole blood), which imparts an undesirable dark color, odor and metallic taste to the final product (Duarte, et al., 1999; X. Q. Liu, Yonekura, Tsutsumi, & Sano, 1996). Their use is restricted also for reasons of hygiene as the cellular fraction was thought to

have a higher microbial load, though this has now been disproved (Nowak & von Mueffling, 2006). Probably due to these undesirable characteristics, RBC concentrates have been banned from the food chain in Germany (Nowak, Heise, Tarnowski, & Von Mueffling, 2007). Despite the predominance of plasma-derived products compared to RBC-derived products, RBC and derived products are used to some extent in the food industry, although they are mainly found in the meat industry as a natural color enhancer in sausage type products (Heinz & Hautzinger, 2007). Compared to plasma derived products, the amount that is used is limited in order to avoid jeopardizing the sensory qualities of the final product; in meat products, their usage is usually restricted to 0.5 to 2% of the product (Slinde & Martens, 1982).

Disposing of the cellular fraction of blood plasma through proper treatment involves considerable cost as disposing of it untreated into sewage and onto agricultural lands is forbidden by law. A valuable source of protein and iron (as heme iron) is also lost if the cellular fraction is not utilized. This has therefore led to efforts to fully utilize the cellular fraction to reduce costs, take advantage of its nutritional value, and avoid the soil and water pollution associated with its disposal. Accordingly, a number of methods have been developed to remove hemoglobin from red blood cells in order to produce the end product known in the industry as "globin" or "decolorized blood", thus making it more useful as a food ingredient. The heme pigment can be removed by extraction with acidified acetone (Tybor, Dill, & Landmann, 1975); by absorption on agents such as carboxy methyl cellulose (Duarte, et al., 1999; Sato, Hayakawa, & Hayakawa, 1981), sodium carboxy methyl cellulose (Autio, Kando, & Kiesvaara, 1984; Yang & Lin, 1996) and sodium alginate (Hayakawa, Matsuura, Nakamura, & Sato, 1986); or by enzymatic hydrolysis (Houlier, 1986). Further processing methods have been devised to produce globin that improves the taste and also have a whiter appearance (Gómez-Juárez, Castellanos, Ponce-Noyola, Calderón, & Figueroa, 1999). Globin, which exhibits good foaming, emulsifying, heat-induced gelation and water

binding capacity, can also be used to replace fat in meat products (Viana, et al., 2005).

Other research has been undertaken to ensure the full utilization of hemoglobin-containing fractions, enabling manufacturers to redeem valuable protein and iron constituents that would otherwise be lost. One example of these proteins that are derived from red blood cells is heme iron polypeptide (HIP), a soluble heme moiety with an attached polypeptide obtained by the enzymatic digestion of bovine hemoglobin. HIP is commercially available in the US and is sold under the brand name Proferrin (<http://www.proferrin.com/index.php>). Studies have shown that supplementing with HIP produces a better iron status by improving iron absorption and enhancing storage (serum ferritin levels) compared to iron salts (Nissenson, et al., 2003). Another example is cooked cured meat product (CCMP), dinitrosyl ferrohemochrome. CCMP is a product synthesized from bovine RBCs for its potential to be used as a meat curing agent (Shahidi & Pegg, 1991; Shahidi, Rubin, Diosday, & Wood, 1985). Traditionally, nitrite is used as a meat curing agent, imparting a characteristic pink color and unique flavor and acting as an antioxidant and antimicrobial agent (Stevanovic & Sentjurc, 2000). However, concerns have arisen due to its potential reaction with amino acids or amines present in the meat, which could lead to the formation of carcinogenic N-nitroso compounds such as N-nitrosoamines. CCMP, which could be a suitable alternative, can be manufactured through either a direct or indirect process (Shahidi, et al., 1985) involving a hemin [iron-containing porphyrin, with iron present in the ferric state (Fe^{3+})] intermediate. Hemin (or heme) is first isolated from RBCs by treatment with acetic acid or acidified acetone to separate the protein portion from the hemin. The recovered hemin crystals are treated with a cocktail of reducing agents and then nitric oxide is introduced into the solution to produce the CCMP, which precipitates out of solution as a result of the reduction in the pH of the medium (Pegg & Shahidi 1997).

The need to avoid consuming blood-derived products

There are groups of individuals in the population, particularly Jews and Muslims, who are required by their religious laws to eat foods that are kosher and halal, respectively. Kosher, which means fit or proper for consumption, is defined by Biblical laws that are enshrined in the original five books of the Bible known as the Torah. Halal foods refer to foods that are lawful or permitted to be eaten by Muslims and are prescribed in the Qur'an. Observant Jews or Muslims must follow these dietary laws, which are considered divine orders; failure to comply will result in divine retribution. Both kosher and halal dietary laws clearly prohibit the consumption of blood, but this prohibition arises from different philosophies. The halal dietary laws are believed to be health inspired, so halal forbids the consumption of blood as it is believed that blood drained from an animal contains harmful microorganisms, toxins and harmful products of metabolism. In contrast, practicing Jews are barred from eating blood as blood is thought to be synonymous with life (Eliasi & Dwyer, 2002). Accordingly, the method given for slaughtering animals according to both the kosher and halal dietary laws is geared towards ensuring that the maximum amount of blood is drained from the carcass.

Individual consumers may also need to avoid consuming blood products because of allergy to blood proteins such as serum albumin and IgG. Bovine serum albumin (BSA), represents the most abundant (50-60%) protein present in the plasma fraction of bovine blood (Davila, Pares, Cuvelier, & Relkin, 2007). BSA is also present in beef and cow's milk and has been implicated in allergic reactions to milk (Goldman, Anderson, et al., 1963; Goldman, Sellars, et al., 1963; Martelli, De Chiara, Corvo, Restani, & Fiocchi, 2002; Wal, 2002) and also to beef (Fiocchi, Restani, & Riva, 2000; Fiocchi, et al., 1995; Han, Matsuno, Ito, Ikeuch, & Suzuki, 2000; Kanny, de Hauteclouque, & Moneret-Vautrin, 1998; Takahata, et al., 2000). Immunoglobulin G, a protein present in both blood and milk has also been reported to be an allergen in cases of meat (Ayuso, et al., 2000) and milk allergy (Maeda, Morikawa, Tokuyama, &

Kuroume, 1993). Others, such as the Seventh Day Adventists, vegetarians and vegans, choose not to eat any animal product or animal blood for a variety of reasons, including religion, personal preference, animal rights, health, and the environmental benefits that derive from eating plants. Yet other people avoid consuming blood products because of their belief that blood contains harmful microorganisms and toxic metabolites that renders them unsafe for consumption.

Protecting consumer interests

According to the Pew Forum on Religion and Public Life, there are 1.57 billion Muslims in the world today, representing about 23% of the estimated global population of 6.8 billion (http://pewforum.org/uploadedfiles/Orphan_Migrated_Content/Muslimpopulation.pdf). The world market for halal foods is valued at \$635 billion per year and demand is increasing in North America (US and Canada), Southeast Asia, Middle East, Europe, North Africa and Australia (http://www.ifanca.org/newsletter/2010_09.htm).

Trade in halal products is reported to be the fastest growing food category in the world (Riaz, 2010). Sales for kosher foods have also risen by about 15% annually over the past decade, although Jews constitute only 20% of the kosher market (Kotz, 2008). Non-Muslims and non-Jews also patronize kosher and halal foods as they have gained global recognition as being both safe and hygienic. There is thus a huge economic incentive for food manufacturers to meet the needs of these consumers. The increase in popularity of kosher and halal foods has, however, created a favorable environment for both deliberate and inadvertent labeling of products that actually violate the respective dietary laws as being kosher or halal. Also, in countries such as India it is estimated that about 35% of the population are vegetarians and have been for many generations, while in the western world the number of vegetarians continues to increase rapidly. Revealing the presence of hitherto undeclared or hidden food ingredients derived from animal blood is therefore of interest to a large sector of the population.

As a myriad of products derived either from the plasma or cellular fraction of animal

blood is becoming commercially available for use in processed food, it is not always clear to consumers that they are of blood origin because generally these blood ingredient products are referred to by their brand names. For example, two dietary products, Daily Immune Defense and Schiff ImmunAssure, both of which contain immunolin® as the active ingredient, lack any indication on the label that immunolin® is in fact derived from bovine blood. Better labeling is therefore vital to protect the interests of those consumers who should avoid such products and also to deter rogue manufacturers who may be tempted to use such products fraudulently. As the use of these blood products continues to increase, effective methods for the detection of their presence in dietary products are also needed.

In order to enforce the food labeling laws, a number of methods have been developed to detect blood and derived products in food as a regulatory tool to address various consumer concerns. Most of these methods have focused on their detection in meat as the meat industry is the greatest user of these products. Spectrophotometric methods based on hemoglobin as the analyte have been developed to detect added blood in raw ground beef (Karasz, et al., 1976; Maxstadt & Pollman, 1980). The Kjeldahl method has also been appropriated to estimate the amount of hemoglobin (and hence blood) in meat products (Bjarno, 1981). Ultra-thin layer isoelectric focusing (Bauer & Stachelberger, 1984) and immunoassay (Otto & Sinell, 1989) have also been developed to detect plasma in meat products. A liquid chromatography tandem mass spectrometric (LC-MS/MS) method based on the detection of fibrinopeptides has also been developed to detect the presence of a commercial bovine (Grundy, et al., 2007) and porcine (Grundy, et al., 2008) plasma-derived food binding agent, Fibrimex®, in food products. Collectively, these methods suffer several drawbacks including being ineffective against heat-treated samples, unable to distinguish between residual blood and added blood, and inaccurate. Recently a panel of monoclonal antibodies (MAbs) which recognize different thermal-stable blood proteins were developed in our laboratories for the detection of different blood

proteins in both raw and processed materials (Hsieh and others 2007). Subsequently, a sandwich enzyme-linked immunosorbent assay (ELISA) using MAbs 3D6 and 6G12 which recognizes a 60kDa serum protein in bovine blood (Ofori & Hsieh, 2007) and a competitive ELISA using MAb 1H9 recognizing a 12kDa cellular protein in ruminant blood (Rao & Hsieh, 2008) have been developed. These two immunoassays overcome the afore-mentioned problems associated with other methods and were initially developed to monitor the presence of added bovine blood material in animal feed for the surveillance of prion diseases such as bovine spongiform encephalopathy. Further testing against a number of commercial food protein ingredients derived from whole blood, plasma and cellular proteins has shown the sandwich and competitive ELISAs to be also effective in the detection and quantification of trace amount of these blood proteins in either raw or processed food matrices (unpublished data). Using these two complementary assays, one for the detection of the plasma proteins and the other for the cellular proteins, to effectively monitor the presence of either plasma or RBC-derived proteins in a wide range of foods has become possible.

Conclusions

In the past, efficient and broader utilization of blood has been hampered by technical problems such as the lack of equipment to hygienically collect the blood, the short shelf life of blood, and the organoleptically unacceptable characteristics of many blood products. Advances in modern technology have surmounted these problems, however, bringing to the market a variety of blood products for use as ingredients in dietary products. Eliminating a sizeable pollution hazard and preventing the loss of a valuable protein source have combined to encourage efforts to better utilize blood and its derived products in the food chain. Notwithstanding these benefits, there remains a need for a way to monitor the use of these products in food for the protection of those who should avoid blood-tainted foods for religious, cultural, ethical or health reasons. Suitable methods that can be used by both regulators and manufacturers to detect the presence

of blood ingredients in foods are vital in order to guarantee the quality of food products and compliance with food labeling laws. The natural tendency for unscrupulous manufacturers to use these inexpensive blood products in dietary products to achieve economic gains only underlines the need for effective analytical methods. Regulatory guidance specifying appropriate labeling for foodstuffs that addresses these issues would serve to boost consumers' confidence in their food choices. Government, academic and industrial scientists should work together to achieve this goal.

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