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Plasma and blood functionality review

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Abstract

Blood is the first co-product obtained while processing animals for meat. Blood-derived co-products have been successfully isolated, characterised and commercialised, and have been used for some time in the food industry in many countries as functional and nutritional ingredients.

Extensive literature and patent searches on the functional properties of blood have shown that blood plasma exhibits a range of functional properties including, water-holding, gelling, emulsification, solubility and foaming capacity. These properties can be influenced by pH, temperature, ionic strength, protein concentration, salts content and processing conditions used to obtain the protein. These aspects are discussed in this report.

From the information gathered, current and potential applications of blood proteins in the food industry have been identified and comparisons to alternative functional food ingredients are discussed. Factors limiting the commercial use of blood components as a food ingredient have also been identified.

Executive Summary

The results of an extensive literature search on the functionality of plasma and blood components and their use as food ingredients has been compiled.

Blood Components

- Blood constitutes approximately 3-5% of an animal's live weight and contains approximately 18-19% protein, of which 7-8% is plasma and 34-38% is red blood cells (RBCs).

- The plasma component of blood is typically 7-8% protein, 91% water and 1% of a variety of salts and low molecular weight compounds. The plasma fraction can be separated into albumin, α, β and γ - globulins, and fibrinogen fractions.

- Blood is a good source of bioavailable iron. Heat processing (cooking) decreases the bioavailability of the iron and absorption of iron is improved when delivered in a meat-based diet: during digestion of meat, ferric iron can be converted into the bioavailable ferrous form.

Functionality of Blood Components

- Blood plasma has been shown to exhibit a range of functional properties including: water holding, gelling, emulsification, and foaming capacity. These properties can be influenced by pH, temperature, ionic strength, protein concentration, salts content and the processing conditions used to obtain the protein.

- Gelling studies have shown that plasma gels are stronger than serum gels due to the influence of fibrinogen during heat-induced gelation.

- pH 7 has been reported to be the optimum pH for gel formation of bovine plasma.

- Plasma protein that has been alkali-treated to pH 11 -11.5 has shown considerable increases in viscosity and good (protein fibre) spinning characteristics at pH 11-13.

- Heating of blood plasma may cause local aggregation of proteins as gels resulting in a coarse structure where moisture is more easily lost. This may be accentuated by higher salt and protein concentrations and can be decreased by adjusting the pH away from the isoelectric point.

- Bovine blood plasma has been reported to have maximum hydrophobicity at pH 3, while maximum emulsifying activity was at pH 7. Protein solubility and emulsifying capacity are relatively constant across the pH range. Addition of salt does not affect emulsion stability but reduces solubility, hydrophobicity and emulsion capacity at pH 5 and 6 and increases emulsifying capacity at pH 5.

- Gamma irradiation of plasma protein powders and solutions from slaughter-house blood does not affect viscosity, protein solubility and secondary protein structure in irradiated powders, but does affect plasma protein solutions causing aggregation of plasma proteins.

- Bovine serum albumin (BSA) appears to be the dominant gel-forming protein of bovine plasma. Maximum gel strength has been reported at pH 6.2 (7 fold greater than at pH 7.8) and could be doubled by raising the temperature from 60 ^{o}C to 74 ^{o}C. Increasing the free fatty acid concentration 10-fold can reduce gel strength by 30% at pH 5.8.

- Blood plasma proteins have been compared to egg white for heat stability, foaming capacity and stability, and emulsification properties. Blood plasma proteins had lower denaturation temperatures than egg white, with gamma globulin the most heat stable and fibrinogen the most heat sensitive proteins. Foaming capacity was similar to egg white, but foam stability was lower while emulsification properties of blood plasma and component proteins were better than egg white.

- Spray dried animal plasma (SDPA) has been reported to have high gel strength capacity above 90^{o}C with a peak gel strength at 121^{o}C, while water

holding capacity of the gel was found to be stable above 80^{o}C. When compared with other binding agents, SDPA was found to have good protein solubility, with better gelling properties than egg albumen, wheat gluten, and porcine protein, but lower gel strength than carrageenan. In a petfood loaf product, SDPA maintained a more compact texture with less exudation when substituted for other binding ingredients at the same level.

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1 Background

Blood proteins have been used for some time in the food industry as functional and nutritional ingredients in many countries, although the use of alternatives in place of blood due to cultural taboos is common in some countries. In food, blood is used in a variety of ways including as a protein supplement, biologically active ingredient, textured meat protein, clarifying agent in liquid foods, as a stabiliser, as a protease inhibitor, as an emulsifier, and as a colouring agent for meat items such as in chicken. Plasma proteins are also reported to provide excellent physiological functional properties associated with health and well being in applications such as meal replacers and in faster muscle recovery products in sports nutrition. Fractions of hydrolysed bovine plasma are reported to provide bioactivity such as angiotensin I converting enzyme (ACE) inhibition related to control of blood pressure.

Whole cattle blood contains 19% solids and 81% water and can be separated into its constituent plasma (9% total solids) and cell (41% total solids) fractions using centrifugation. The plasma fraction has several functional properties which increase its value. These include heat-set gelation (to form a bland-tasting white gel), emulsifying (fat binding) capacity and water holding capacity. Plasma has been used as a natural ingredient in the production of smallgoods because of these inherent emulsifying and water holding properties. Whilst casein as a protein is superior to blood in this application, soy and meat proteins are inferior to blood. Blood plasma also has potential in bakery products as a foaming agent and leavening agent. Blood albumin is used as an egg albumen substitute, in making sausage casings and in bread flour extension. Blood haemoglobin is useful not only as a colouring agent but also as a catalyst in oxidation of unsaturated fatty acids in moderate concentrations. When separated from the haem, the globin protein in the red cell fraction is reported to provide excellent gel strength and surface active properties.

Therefore, blood has a broad and integral use in the food industry. However, there are alternative non-blood ingredients that compete with blood in the different functional applications in the food industry.

2 Project Objectives

Conduct a comprehensive review of the scientific and technical literature on the functional properties of blood, its applications, and the current use of alternative (non-blood) ingredients to achieve functional properties in the food processing industry, including:

- A description of functional properties of blood constituents reported in the scientific literature
- A description of the potential applications of blood to achieve functional properties in the food industry as reported in the scientific and technical literature
- A description of the current use of blood by the global food industry to achieve functional attributes in processed foods
- Identification and description of ingredients that are currently used by the food industry to achieve functional properties that could also be achieved by substitution with blood constituents
- A description of market information for each of the functional categories that blood could potentially be substituted in the food industry
- Identification of the limiting factors/characteristics of blood in comparison to alternative functional food ingredients

This report will provide a synthesis of information on blood characteristics and functional properties and applications, and a comparison to alternative functional food ingredients. This report can be used as a basis for developing strategies for the processing and marketing of blood-derived functional ingredients.

3 Methodology

Information from literature and patent searches on the functionalities of blood in the world food industry and alternative competitive ingredients was sourced via detailed and thorough strategic electronic searches of scientific and technical literature.

Scientific Literature

Searches were carried out on Food Science and Technology Abstracts (FSTA) and Commonwealth Agricultural Bureau International (CABI) Abstracts on 23rd and 28th of March, 2011. These are the two foremost databases with both having extensive coverage of the literature of the meat and livestock industry and food ingredient industry. The specific coverage of these two databases is provided below.

Food Science and Technology Abstracts or FSTA is the internationally recognised world's leading Food Science and Technology Abstracts database. Covers material published since the late 1960s.

Commonwealth Agricultural Bureau International Abstracts or CABI contains over 3.8 million records from over 10,000 journals, books, conferences, reports, and other kinds of literature published internationally. Subjects covered include animal and crop husbandry, animal and plant breeding, plant protection, genetics, forestry, economics, veterinary medicine, human nutrition, and rural development. This database indexes all significant worldwide publications in meat science.

These two databases were selected because of their complete coverage of the literature of both the food and meat industries. It was felt that a combination of these two would be most likely to supply not only the scientific material but also any significant achievements announced in the trade literature.

A list of relevant keywords for blood proteins and functional properties was compiled by the team members for use against these major food databases. These terms were then converted to the specific descriptor or key word index forms as shown in the two database's thesauri or hierarchies of terms. This restricted the results to items where the required subjects were significant enough to be indexed and not just concepts mentioned in passing.

CABI Abstracts (Searched by Christine Margetts)
The following descriptors were chosen from the CABI descriptor hierarchy and combined.

Animal Blood Terms
Blood and protein*
blood proteins
fibrinogen
albumin
immunoglobulin*
haemoprotein* or hemoprotein*
"red cell concentrate*"

Functional Properties Terms
functional properties or chemico-physical properties or physicochemical properties or physico-chemical
emulsification
gelation
foams or foaming
solubility or water activity
binding
buffering
whipping or whipping agents
dispersability

Broad adjunct searches to double check the results were also carried out using:
"red cell" and protein* and properties
(serum or plasma) and protein*

FSTA (Searched by Christine Margetts)
The following descriptors were selected from the FSTA thesaurus and combined with the broad and narrow descriptors for functional properties shown below. Some terms, such as the names of specific blood proteins were free text searched as they would only show up in the record abstract.

Animal Blood Terms
Blood proteins
proteins animal
albumins
globulins
protein isolates
bovine serum albumin
animal proteins
fibrinogen
haemoglobin

Functional Properties Terms
Functional properties
hydrophobicity
interfacial tension
ionic strength
binding capacity

buffering capacity
dispersibility
emulsification properties or emulsifying capacity
foaming or foaming agents or foaming properties
gelling capacity
pasting properties
surface active properties or swelling or water binding capacity or water holding capacity
whipping properties or whipping capacity
solubility or solubilization
stability or colloidal stability or stabilization

These searches were then restricted to the FSTA section of “Meats Poultry and Game”.

(Blood or serum or plasma) and protein* and functional properties was also searched more broadly as descriptors to double check the results

Relevant search results were loaded onto an Endnote Database and transferred to other team members.

Science Citation Index Expanded (Searched by Raymond Mawson)
Additional searches on protein functionality were carried out on Science Citation Index Expanded part of Web of Knowledge. This database covers 6650 scientific journals including major peer-reviewed food journals. Search strategy was devised by Raymond Mawson, whose brief was to examine foaming in particular and focussed particularly on the period since 1978 when previously involved in blood plasma applications research.

Generic search
Blood plasma

Modifiers used in combination with the original generic search – individually and in combinations.
Functionality
Review
Foam and related terms
Bovine
Globulin
Serum albumin
Modification and related terms

Based on Raymond’s pre-existing knowledge from experience in the field, critical review papers and any especially relevant foaming papers in particular, were used to seed citation search trees that were selectively searched.

The most relevant papers were collected into a Refman 11 database of approximately 100 references and the papers obtained for closer examination. Notes

on the papers were compiled in Refman using Dragon 10 dictation software and used as the basis for the review.

Patent Literature (Searched by Christine Margetts)
Intellectual property searches were carried out on Derwent Innovations Index, from 18-22 March, 2011, a respected patent database with worldwide coverage. Derwent covers material back to 1963 although this coverage varies with the country of origin (see Appendix 1). This variable country coverage means that material that has been registered in a single jurisdiction prior to that jurisdiction being covered by Derwent will not appear in these results.

The advantage of Derwent, apart from its breadth of coverage, is that it collates patents into their families. This means that the user does not have to bring single applications in different countries for the same patent together in order to follow their registration and status in different patent jurisdictions. If a patent was registered later in a covered zone, the patent family approach sometimes mitigates the country coverage variability issue.

The following bolded International Patent Classification (IPC) codes, shown in their hierarchy of meaning, were used for the initial searches. These codes substitute for keyword or descriptor indexing on patent databases.

A23 - FOODS OR FOODSTUFFS; THEIR TREATMENT, NOT COVERED BY OTHER CLASSES
A23J - PROTEIN COMPOSITIONS FOR FOODSTUFFS; WORKING-UP PROTEINS FOR FOODSTUFFS; PHOSPHATIDE COMPOSITIONS FOR FOODSTUFFS
A23J-001/00 - Obtaining protein compositions for foodstuffs; Bulk opening of eggs and separation of yolks from whites
A23J-001/06 - from blood

A23J-003/00 - Working-up of proteins for foodstuffs A23J-003/04 - Animal proteins
A23J-003/12 - from blood

Free text searches were carried out using the following concept sets.

Animal Blood Terms
bovine serum albumin or BSA
Bovine blood
Animal blood
Blood and ingredient*
Blood plasma
Blood serum protein*
Blood plasma protein*
Blood plasma fraction*

Blood protein isolate*
Blood protein plasma fraction*
Blood globin
Blood globulin
Blood albumin
Serum gel*
Serum protein*
Plasma protein*
Plasma fraction*
Protein isolate*
Fibrinogen
Haemoprotein* or hemoprotein*
Haemoglobin or hemoglobin
Red cell concentrate

The broad terms blood or plasma or serum sometimes were also combined with bovine or porcine or galline or ovine to refine the results.

Functional Properties Terms
Water holding or water binding or water retention or solubility or solubili* or swelling or dispersability or dispersal
Emulsifi* or emulsion* or emulsify
Textur*
Leavening or leavened
Precipit*
Whipped or whipp*
Foam or foaming or paste or pasting
Gel or gelled or gel-forming or gelation
Binding or buffer or swelling or stabilise* or stabilize
Surface active or surface activity

All searches were also further restricted to food applications using the bolded Derwent Class Code Hierarchy codes from the Chemical Sections.

D - Food, Detergents, Water Treatment and Biotechnology
D1 - Food and Fermentation
D11 - Baking - including bakery products, flour, doughs, bakery ovens, dough transporting and/or handling equipment, pies and pasta, but not flour milling. (A21).
D12 - Butchering, meat treatment, processing poultry or fish. (A22).
D13 - Other foodstuffs and treatment - including preservation of food, milk, milk products, butter substitutes, edible oils and fats, non-alcoholic beverages, artificial sweeteners, food additives and animal feed. (A23B A23C A23D A23E A23F A23G A23H A23I A23J A23K A23L).
D14 - General foodstuffs machinery - excluding machines which can be classified in D11- 13
D17 - Sugar and starch industry. (C07H C13).

Code D16 - Fermentation industry - including fermentation equipment, brewing, yeast production, production of pharmaceuticals and other chemicals by fermentation, microbiology, production of vaccines and antibodies, cell and tissue culture and genetic engineering was initially included but produced a multitude of records on test kits and other materials that used blood proteins and so it was taken out to reduce sets to manageable results.

The results of these searches were hand combed or relevance and selected sets downloaded onto an Endnote database and supplied to the project teams. Many of the resulting sets were large, in the case of broad terms or very small in the case of very specific narrow terms and so selections for transfer were cumulated over each day's searching, thus no set result numbers are shown here. The resulting database was supplemented by additional records from Web of Knowledge Science citation Index from additional searches carried out by another team member.

Country Coverage of Derwent Innovations Index.

Although Derwent Innovations Index, covers material back to 1963 this coverage varies with the country of origin (Table 1). This variable country coverage means that material that has been registered in a single jurisdiction prior to that jurisdiction being covered by Derwent will not appear in the search results.

Table 1 : Country Coverage by Derwent Innovation Index

Country/Abbreviation	Initial Year of Coverage
Argentina (AR)	1974-1976 only
Australia (AU)	1983 (also 1963-1969)
Austria (AT)	1975
Belgium (BE)	1963
Brazil (BR)	1976
Canada (CA)	1963
China (CN)	1987
Czech Republic (CZ)	1994
Czechoslovakia (CS)	1975-1994
Denmark (DK)	1974
European Patents (EP)	1978
Finland (FI)	1974
France (FR)	1963
Germany (East) (DD)	1963
Germany (DE)	1963
Hungary (HU)	1975
India (IN)	2004
International Technology Disclosures (TP)	1984-1993
Ireland (IE)	1963-1969; 1995
Israel (IL)	1975
Italy (IT)	1966-1969 (section A subjects only); 1978
Japan (JP)	1963
Korea (KR) (South)	1986
Luxembourg (LU)	1984
Mexico (MX)	1997

Country/Abbreviation	Initial Year of Coverage
Netherlands (NL)	1963
New Zealand (NZ)	1993
Norway (NO)	1974
Patent Cooperation Treaty (WO)	1978
Philippines (PH)	1995
Portugal (PT)	1974
Research Disclosure (RD)[1]–	1978
Romania (RO)	1975
Russian Federation (RU)	1994 (Russia)
Singapore (SG)	1995
Slovakia (SK)	1994
South Africa (ZA)	1963
Soviet Union (SU)	1963-1994
Spain (ES)	1983
Sweden (SE)	1974
Switzerland (CH)	1963
Taiwan (TW)	1993
United Kingdom (GB)	1963
United States (US)	1963

4 Results

4.1 Composition of blood

Blood accounts for ~3-5% of an animal's live weight and contains ~18-19% protein, similar to that found in lean meat, with the protein contents of plasma and red blood cells (RBC) being ~7-8% and 34-38%, respectively.

The most abundant protein in the plasma fraction is albumin followed by globulin and the third most abundant protein is fibrinogen (Table 2). Haemoglobin is the conjugated protein in red blood cells. It consists of four polypeptide chains arranged in a spherical structure with a haem group, which is a ferrous protoporphyrin group embedded in the structure.

Table 2: Concentration of blood components in plasma

Component	**Concentration in plasma mg/100mL**
Plasma	
Albumin fraction	3000-4500
Globulin fraction	
• α1- globulins	100
• α2- globulins	400-900
- α2 glycoproteins	-
- ceruloplasmin	30
- prothrombin	-
• β - globulins	600-1200
- β1 lipoproteins	350-450
-transferrin	40
-plasminogen	-
• γ- globulins	700-1500
α1- lipoproteins	350-450
Fibrinogen fraction	300
Red blood cells	
Globin	

4.2 Functional properties of blood components

Animal blood is a rich source of proteins which can be used as functional food ingredients in formulated food products as an alternative to traditional proteins and other surfactants. Over the last four decades, several studies have been conducted on the functional properties in model systems of blood-derived proteins originating from bovine, porcine, equine, galline (chicken) and other animal species. The most regularly investigated functional properties are solubility, emulsifying, foaming and gelling properties. Apart from their intrinsic molecular characteristics, the functional properties of proteins are dependent on extrinsic factors such as methods of isolation, pH, ionic strength as well as interactions with other components (Kinsella, 1981; Damodaran, 1994;). Thus many of the studies are on the effect of these different parameters on the functional properties of blood proteins.

Solubility

Solubility is an important functional property of proteins as it influences other functional properties. Proteins that are used to impart physical functionality need to have high solubility (Ramos-Clamont et al., 2003). The solubility of proteins at different pH, ionic strength and temperature conditions gives a good indication of the potential applications of proteins (Alvarez et al., 2009). There are several studies in the literature on the solubility of dried whole plasma, globulin, albumin and globin at different pH and ionic strength conditions. The method of drying might also affect the functionality of the blood proteins.

4.2.1.1 Whole plasma

The solubility of plasma (proteins) is quite high ranging from 75 to 100% over a wide range of pH (3.0-8.0) (Vuono et al., 1979, Silva and Silvestre, 2003, Davila et al., 2007c, del Hoyo et al., 2008). The effect of pH on the solubility of plasma is limited. Vouno et al. (1979) reported that freeze dried bovine plasma showed maximum solubility of 100% at pH 3.0 and pH 6.0-8.0. The minimum solubility of 75% was observed at pH 5.0 (Vuono et al., 1979). Silva and Silvestre (2003) observed the solubility of bovine plasma to range between 70 and 80% at pH between 3.0 and 8.0, with no substantial effect of pH, although slightly higher solubility was observed at pH 4.0. Similarly, Davila et al. (2003) reported that the solubility of porcine plasma was about 85% at pH between 4.5 and 7.5, with a very slight effect of pH. del Hoyo et al. (2008) observed solubility of porcine plasma between 68 and 80% at pH between 3.0 and 8.0, with slightly lower values at pH 6.0. In contrast, a significant reduction in the

solubility of chicken plasma was observed at pH around 5.0, which was more pronounced in dialysed samples (with reduced low MWt components). The solubility of the untreated and the dialysed samples decreased from about 98% at pH 4.0 to 58% and 10% respectively at pH 5.0. Further increase in pH resulted in improved solubility and the solubility was about 95% at pH 8.0 for both samples (del Rio de Reys et al., 1980). Ionic strength also influences the solubility of plasma to a certain extent. Silva and Silvestre (2003) observed about 10% reduction in the solubility of bovine plasma in the presence of 0.035 M NaCl at pH 5.0 and 6.0 (Silva and Silvestre 2003). In contrast a significant increase in the solubility of dialysed and untreated chicken plasma was observed in the presence of 0.1 to 0.5 M NaCl, increasing the solubility of dialysed and untreated plasma by 70% and 30% respectively at pH 5.0, where the lowest solubility was observed (del Rio de Reys et al., 1980).

Apart from pH and ionic strength, the solubility of plasma has been reported to be influenced by the method of drying. Tybor et al. (1973) compared the effect of freeze drying with spray drying at different temperatures on the solubility of bovine plasma. Drying method had significant effect on the solubility of plasma. Samples spray dried at 160˚C and 193˚C had about 20% less solubility compared to freeze dried samples. The effect of spray drying temperature was not significant. All samples exhibited reduced solubility around pH 5.0 as observed in other studies. Addition of lactose significantly improved the solubility of spray dried samples with about 20% in the case of samples dried at 160˚C and about 10% in the case of samples dried at 193˚C.

Processes such as demineralisation and enzymatic hydrolysis have significant impact on the solubility of plasma. del Hoyo et al. (2008) reported that demineralisation of porcine plasma by ultrafiltration improved the solubility of plasma by a maximum of 10%, whereas decationisation by ion exchange decreased the solubility by about 15%. This was attributed to changes in net charge due to the removal of cations affecting protein-water interaction (del Hoyo et al., 2008). Rodriguez-Furlan (2010) compared the solubility of spray dried bovine plasma powder with that of the same powder that had undergone ultrafiltration and freeze drying in the presence of 12% sucrose as a stabiliser. The treatment resulted in a significant increase in the solubility of the plasma powder. The difference in solubility was about 10% at pH 3.0 and 13% at pH 7.0. The processed plasma had about 85% solubility whereas the raw plasma had about 72% solubility at pH 7.0. Both showed minimum solubility at pH 5.0 to 6.0, and maximum solubility at pH 8.0 (Rodriguez-

Furlan et al., 2010). Silva and Silvestre (2003) reported that partial hydrolysis of bovine plasma by trypsin significantly reduced its solubility. The effect was dependent on the processing conditions. For samples that were hydrolysed for 15 to 60 min, the reduction in solubility was not significant; while up to 40% reduction in solubility was observed in samples that were hydrolysed for 5 to 10 minutes. Significant reduction in the solubility of all the hydrolysed samples was observed at pH 5.0 to 8.0, although the reduction in solubility was less in samples subjected to 15 to 60 minutes hydrolysis (Silva and Silvestre 2003). High pressure processing (450 MPa, 15 min, 5°C and 40˚C) did not have a significant impact on the solubility of porcine plasma at pH 7, while the solubility at pH 4.5 was reduced by about 10% and 20% respectively after high pressure processing at 5°C and 40˚C (Pares et al., 2000).

4.2.1.2 Serum

The solubility of porcine serum has been reported to be > 85% over a wide range of pH (Ramos-Clamont et al., 2003; Davila et al., 2007c). Ramos-Clamont et al. (2003) observed a significant effect of pH on the solubility of porcine serum, with an increase in solubility from 70% to 92% as the pH increased from 5.0 to 8.0. In contrast, Davila et al. (2003) reported only a slight (~2%) increase in the solubility of porcine serum as the pH increased from 6.0 to 7.5, with no significant difference between pH 4.5 and 6.0.

4.2.1.3 Albumin

The reported values for the solubility of albumin range from 70% to 100%, with no significant effect of pH on the solubility of albumin of both bovine and porcine origin (Davila et al., 2007b; Ramos-Clamont et al., 2003; Vuono et al., 1979) On the other hand, ionic strength slightly affects the solubility of albumin. Ramos-Clamont et al. (2003) reported that the solubility of porcine albumin decreased with increase in ionic strength, with about 8% reduction in solubility in the presence of 0.25 M phosphate buffer.

4.2.1.4 Globulin

The solubility of globulin ranges from 40 to 90% depending on the source, pH and ionic strength. Vouno et al. (1979) observed a maximum of 90% solubility of bovine globulin at pH 8.0., whereas the lowest solubility of about 40% was observed at pH 5.0 to 6.0 (Vuono et al., 1979). Ramos-Clamont et al. (2003) observed a significant

increase in the solubility of porcine globulin with an increase in pH from 6 to 8 and increase in ionic strength from 0 to 0.25 M phosphate buffer. Solubility increased from about 62% to 75% as the pH increased from 6.0 to 8.0. About 10% increase in solubility was observed with increase in ionic strength to 0.25 M phosphate buffer at pH 6.0, while the increase was about 15% at pH 8.0 (Ramos-Clamont et al., 2003).

4.2.1.5 Fibrinogen

There are not many studies on the solubility of fibrinogen. Alvarez et al. (2009) reported that the solubility of porcine fibrinogen is very low (<20%) in the pH range of 3.0 to 8.0.

4.2.1.6 Haemoglobin and globin

Most studies on the functional attributes of the blood proteins in the cell fraction are on globin, since the application of haemoglobin in human food is limited due to the intense colour of the haem pigment and the special 'blood taste' (Wismer-Pedersen 1988). The solubility of porcine haemoglobin varied between 75% to 95%, the lowest value corresponding to pH 5.0 (Alvarez et al., 2009). Haemolysed red blood cell fraction of porcine blood showed around 98% solubility at pH 4.5 and 7.0. High pressure processing (400 MPa, 15 min) reduced the solubility by about 10% (Toldra et al., 2008).

The solubility of globin significantly depends on the methods employed for isolation and separation from the haem fraction as well as pH and ionic strength (Alvarez et al., 2009; Wismer-Pedersen 1988). Several processes have been developed over the years to separate haem from the globin protein including removal of haem with acidified organic solvents such as acetone, by absorption with active carbon or carboxymethylcellulose (CMC), by protein partial hydrolysis using enzymes or acids, and through oxidative destruction of haem using hydrogen peroxide, sodium hypochlorite and other compounds. The isolation of globin from haemoglobin makes it more labile to denaturation and it may be denatured by the organic acids or the acid pH used for isolation. This significantly reduces its solubility in the pH range between 6.5 and 9 and further reductions occur in the presence of NaCl, limiting its application in product formulation (Wismer-Pedersen 1988).

Vouno et al. (1979) reported that the solubility of globin fractionated by extraction with acidified acetone ranged from 50% to 60% at pH between 3.0 and 8.0. The minimum solubility of 50% was observed at pH 5.0 to 6.0. Maximum solubility of globin was observed at pH 3.0 and 8.0 (Vuono et al., 1979). Tybor et al. (1975)

reported about 90% solubility at pH 3 to 6 for globin prepared by the acidified acetone method, which is higher than reported by Vouno et al. (1979) in the same pH range. The solubility decreased substantially to about 20% at pH 7. The drying methods (freeze drying versus spray drying) and spray drying temperature did not have significant impact on the solubility of globin (Tybor et al., 1975). A similar solubility profile was reported by other researchers for globin isolated in the same way (Shahidi et al., 1984). Gomez-Juarez et al. (1999) prepared bovine globin by enzymatically hydrolysing haemoglobin followed by oxidation of the haem group using sodium hypochlorite. The highest solubility of globin was observed at pH 10 (90%) and the lowest at pH 4.0 (10%). The solubility between pH 6.0 and 7.0, was about 80%, indicating that the solubility of globin is dependent on the method of preparation (Gomez-Juarez et al., 1999). Silva et al. (2003) compared the solubility of bovine globin fractions produced by the acidified acetone method and the CMC absorption method with that of sodium caseinate. Globin extracted by acidified acetone showed the highest solubility of 90% at pH 6 (the pH most relevant to processed meat products) whereas the CMC extracted globin had 40% solubility. The solubility of sodium caseinate at the same condition was 8%. The solubility of sodium caseinate increased in the presence of 0.025 M NaCl, while the solubility of CMC extracted globin decreased to about 30%. The solubility of all the proteins decreased substantially when the NaCl concentration increased to 0.25 M, the concentration of salt relevant to meat products. The highest solubility in the presence of 0.25 M NaCl of about 8% was observed for the CMC extracted globin followed by sodium caseinate at 5% (Silva et al., 2003).

Autio et al. (1984) studied the effect of pH on the solubility of spray-dried porcine globin isolated by the CMC adsorption method. Maximum solubility close to 100% was observed at pH 3.0 to 4.0 and pH 11.0 to 12.0. The solubility decreased significantly in the pH range from 5.0 to 8.0, which is most relevant to food systems. The minimum solubility of about 10% was observed at pH 7.0 to 8.0. The solubility was significantly reduced in the presence of 0.5 M NaCl, while globin was insoluble in the presence of 1 M NaCl at pH 3 to 11. The solubility was around 20% in the pH range from 4.0 to 10.0 in the presence of 0.5 M NaCl (Autio et al., 1984). Alvarez et al. (2009) compared the solubility of two porcine globin fractions; coloured globin obtained by precipitation of haemoglobin by ethanol and another fraction that was subjected to further decolourisation using H_2O_2. The solubility of the decolourised fraction decreased with increase in pH from about 85% at pH 3.0 to 15% at pH 8.0. The coloured fraction exhibited significantly higher solubility ranging from 55% to

98% at pH 3.0 to 8.0. It had the highest solubility of 98% at pH 3, which decreased to about 55% at pH 4.0 and increased with further increase in pH to about 85% at pH 8.0 (Alvarez et al., 2009). Lima et al. (2010) compared the solubility of bovine globin subjected to heating for 30 min at 45˚C and freeze dried with that of globin gel prepared by heating globin for 30 min at 60˚C. The globin was prepared by the acidified acetone extraction. No difference was observed in the solubility of the two preparations. However the solubility of both significantly decreased at pH 7 to about 50% compared to about 80% to 90% at pH 5 and 6 (Lima et al., 2010).

Emulsifying properties

The main parameters that are commonly used to characterise the emulsifying properties of food materials are emulsifying capacity (EC), emulsifying activity, emulsifying activity index (EAI) and emulsion stability. Emulsifying capacity is measured as the maximum amount of lipid emulsified by a liquid dispersion. Emulsion activity index is the area of the oil-water interface stabilised per unit weight of protein and measures the capacity of the protein to stay at the water oil interface after the formation of the emulsion. Emulsion stability is the maintenance of a homogenous structure and texture of a system over a period of time (Mangino, 1994). Emulsion capacity and emulsion activity index reflect the ability of a protein to aid the formation of an emulsion, while emulsion stability reflects the ability of the protein to impart strength to emulsion for resistance to stress (Rodriguez-Furlan et al., 2010). There are no standard methods for the measurements of these parameters (Damodaran, 1994). Thus quantitative comparison of the data from different laboratories is difficult, since there is a wide discrepancy even in the values reported by different laboratories which use apparently the same methods.

Vouno et al. (1979) studied the composite emulsifying activity and stability of bovine blood fractions in the presence of 0.075% NaCl, expressed as the percentage of oil that stayed emulsified after whipping, heating at 70˚C and centrifugation. Whole plasma showed the highest emulsifying capacity (100%) at 0.2% concentration, which was followed by globulin (60%), albumin (25%) and globin (20%) in that order. Protein concentration did not have significant effect on the emulsifying capacity of plasma, although about 20% reduction was observed at 0.8% concentration and higher. The emulsifying capacity of globin and albumin increased to about 30% and 40% respectively, as the concentration increased from 0.2% to 1%. The emulsifying capacity of globulin increased to 100% as concentration increased to 0.8%, followed by a decrease to about 60% at 1% concentration. The emulsifying capacities of

plasma and globulin were comparable with that of soybean protein isolate (Vuono et al., 1979). Similar results were reported by Alvarez et al. (2009) for porcine blood proteins fractionated into different components. At the same protein concentration, fibrinogen showed the highest emulsion capacity, followed by plasma, γ-globulin, α- and β-globulin, albumin, haemoglobin and globin in that order. Concentration between 0.2% and 1% did not have significant effect on the emulsifying capacity of plasma and fibrinogen while the emulsifying capacities of haemoglobin and globin (coloured and decolourised) increased with concentration (Alvarez et al., 2009).

Prata and Sgarbiere (2008) compared the emulsifying capacities of bovine serum albumin (BSA) and globulin at pH 3 to 7 and salt concentration between 0 and 0.15 M. The emulsifying capacity of BSA decreased with increase in pH while the opposite was observed for globulin. The emulsifying capacities of both proteins decreased in the presence of salt except at pH 7.0, where higher emulsifying capacity was observed in the case of BSA at 0.15 M salt concentration. The emulsifying capacity of BSA was higher than that of globulin at pH 3.0 and pH 5.5, whereas the opposite was observed at pH 7.0. On the other hand, the emulsion stability and the emulsion stability index of globulin was better than that of BSA at pH 5.5, which had similar emulsion stability as whey protein concentrate (Prata and Sgarbieri, 2008). A slightly different trend was reported for porcine plasma proteins by Davila et al. (2007c) who compared the emulsifying activity index (EAI) of porcine plasma and its constituents including serum, albumin and globulin at pH 4.5, 6.0 and 7.5. All the proteins showed good EAI with a similar range of values. The effect of pH on EAI in the studied range was minimal. Plasma had a slightly lower EAI at pH 7.5, while albumin had a slightly higher EAI at pH 6.0. Significant differences were observed in the emulsion stability index (ESI) of the different proteins at different pH. The highest emulsion stability was observed in the case of albumin followed by globulins at pH 7.5. Albumin also showed higher emulsion stability at pH 6.0 than all the fractions investigated. In all cases, significantly higher emulsion stability was observed at pH 6.0 and 7.5 compared to pH 4.5 (Davila et al., 2007c). Likewise, Silva and Silvestre (2003) observed no effect of pH from 3.0 to 8.0 on the emulsion capacity of bovine plasma, although a significant increase was observed in the EAI at pH 7.0. On the other hand, the emulsion capacity of plasma decreased by about 90% in the presence of 0.035 M NaCl at pH 5.0 and 6.0. The effect on EAI was pH dependent with about 30% increase in EAI in the presence of 0.035 M NaCl at pH 5.0 and 50% reduction at pH 6.0. The emulsion stability of plasma was reported to be very low at pH 5.0 and 6.0 both at 0 and 0.035 M of NaCl (Silva and Silvestre 2003).

The emulsifying properties of globin are dependent on pH, ionic strength and the method of isolation. The emulsifying activity, emulsion stability and emulsion capacity of globin, prepared by enzymatic hydrolysis of haemoglobin and oxidation by sodium hypochlorite, increased with increasing pH from 2.0 to 10.0. The protein showed good emulsifying activity and stability (Gomez-Juarez et al., 1999). The opposite was observed by Lima et al. (2010), who reported a significant decrease in emulsion capacity and stability of freeze dried and gelled globin preparations isolated by the acidified acetone method, as the pH increased from 5.0 to 7.0. Both preparations had the best emulsifying properties at pH 5.0 (Lima et al., 2010). The difference might be due to differences in the method of isolation. Silva et al. (2003) compared the emulsifying properties of bovine globin isolated by acidified acetone and CMC methods. The emulsifying capacity of the CMC isolated globin was higher than that of the acidified acetone isolated globin at pH 6.0. The opposite was observed with respect to the emulsifying activity index. No significant difference was observed in the emulsion stability. Significant decreases in emulsifying capacity and emulsifying activity index of both isolates occurred in the presence of 0.025 to 0.25 M NaCl, while the emulsion stability remained constant. No difference in emulsifying capacity was observed between the two isolates in the presence of salt, while the emulsifying activity index was higher in the case of the acidified acetone isolate. The authors compared the emulsifying properties of the two globin isolates with that of sodium caseinate. Sodium caseinate had the least emulsifying capacity in the absence of NaCl, while it exhibited higher emulsifying capacity at 0.025 and 0.25 M NaCl. The emulsifying activity index was less than that of the acidified acetone globin isolate at all conditions, while its emulsion stability was higher at 0.25 M NaCl (Silva et al., 2003). Miyaguch et al. (1992) compared the emulsifying capacity of globin with porcine plasma, casein, soy protein and egg white. They noted that globin had high emulsifying capacity at pH 1.0 to 6.0, which was as high as plasma, casein, soy protein and egg white at pH 3.0, although its emulsifying capacity decreased significantly with increase in pH and in the presence of 3% NaCl. Combination of globin with plasma (2:1) improved the emulsifying activity of globin at higher pH (Miyaguchi et al., 1992). Nakamura et al. (1984) compared the emulsifying activity of bovine globin isolated by the CMC method with that of BSA, haemoglobin and ovalbumin at pH 3.0 to 9.0. BSA had the highest emulsifying activity followed by globin, haemoglobin and ovalbumin, in that order. A significant decrease in the emulsifying activity of globin and haemoglobin occurred around pH 7.0, in agreement with the previous study (Nakamura et al., 1984). Waniska et al. (1981) compared the emulsifying activities of bovine serum albumin (BSA) with other food proteins

including ovalbumin, β-casein, β-lactalbumin and soy protein isolate at pH 6.5. BSA showed superior emulsifying activity compared to all the other proteins investigated with relative emulsion activity of 1.0, 0.32, 0.30, 0.24 observed for BSA, ovalbumin, β-casein, β-lactalbumin and soy protein isolate respectively (Waniska et al., 1981).

Physical and biochemical processing of blood proteins affect their emulsifying properties. Bovine serum albumin, as a major protein in plasma, was studied as a model protein to understand the effect of enzymatic hydrolysis on the emulsifying properties of plasma proteins. BSA was hydrolysed by pepsin and trypsin at different conditions. Samples partially hydrolysed by trypsin at 1:100 ratio at 37˚C showed a significant increase in emulsifying activity, with about 40% increase after 60 min hydrolysis. Tryptic hydrolysis for up to 2 hrs improved the emulsifying activity, although further hydrolysis resulted in a significant reduction. No increase in emulsifying activity was observed with peptide hydrolysis (Saito et al., 1993). Tryptic hydrolysis of bovine plasma reduced the emulsifying capacity of plasma at most hydrolysis conditions, while it had no significant effect on emulsifying activity index and emulsion stability (Silva and Silvestre 2003). On the other hand, tryptic hydrolysis increased the emulsifying capacity of bovine globin at pH 3.0 to 8.0 and EAI at pH 4.0. Tryptic hydrolysis of globin for 60 min also increased the emulsion stability at pH 7.0 (Ornellas et al. 2001). Similar results were reported by Bizzoto et al. (2005). Lima et al. (2010) compared the emulsifying properties of freeze-dried globin with that of gelled globin. No significant differences in emulsifying capacity, emulsifying activity index and emulsion stability were observed between the two preparations (Lima et al., 2010). Similarly, drying method (freeze drying versus spray drying) did not have a significant effect on the emulsifying capacities of bovine albumin and globulin at pH 3.0 and 5.5. On the other hand, freeze-dried globulin had a slightly higher emulsifying capacity at pH 7.0 compared to spray dried globulin (Prata and Sgarbieri, 2008)

The emulsifying properties of untreated bovine plasma with decationised and demineralised plasma were compared (del Hoyo et al., 2008). The decationised plasma had the highest emulsifying capacity at higher concentration (6 g/L to 10 g/L) at pH 6.5 (del Hoyo et al., 2008). Rodriguez-Furlan (2010) studied the effect of ultrafiltration and freeze drying in the presence of 12% sucrose on the emulsifying capacity and stability of spray dried plasma powder. The emulsion capacity of the processed powder was higher than that of the untreated powder at all pH values. The maximum emulsion capacity was observed for the processed plasma powder at pH 7.0. A significant reduction in the emulsion capacity of the untreated plasma powder

was observed at pH values higher than 6.0, whereas no such decrease was observed in the case of the processed plasma (Rodriguez-Furlan et al., 2010).

Foaming properties

The foaming behaviour of food proteins is of significant importance to food systems such as whipped cream, soufflés, mousses, ice cream, meringues and leavened bread, since they are formed by the entrapment of air which is enveloped by protein films (Kinsella, 1981). Foaming capacity and foam stability are commonly used to characterise the foaming properties of food materials. Foaming capacity is measured as the amount of foam formed per unit volume of solution and represents the interaction between the liquid solutions and air. Foam stability represents the period of time in which the foam remains stable. Both foaming capacity and the stability of the foam in the presence of other food ingredients at different pH, and during heating are important criteria by which the foaming properties of proteins are characterised (Kinsella, 1981). Due to lack of standardised methods, meaningful comparison of numerical values reported by different laboratories is quite difficult as in the case of emulsifying properties.

Vuono et al. (1979) compared the foaming capacity and foam stability of bovine blood components. Globin showed the highest foaming capacity followed by albumin, globulin and plasma in that order at pH 4.0 to 8.0. Albumin, globulin and plasma (total protein) exhibited the same foam stability, while globin showed very poor foam stability. Overall, albumin showed the best foaming property. The foaming capacity and stability of albumin and globulin was not affected by change in pH between 4.0 and 8.0, while a slight increase in the stability of the globin foam was observed at pH 8.0. On the other hand, the foaming capacity of plasma increased 2.5 fold with increase in pH from 4.0 to 8.0, without any effect on foam stability (Vuono et al., 1979). Similarly, Tybor et al. (1973) observed significantly higher foaming capacity for bovine globin compared to plasma and egg albumen at the same protein concentration. The foaming capacity of plasma decreased with pH increase from pH 2.0 to 10.0. A substantial decrease in the foaming capacity of globin occurred at pH 7.0 corresponding to the commonly observed pH of lowest solubility, although the foaming capacity was still higher than that of plasma at pH 7.0. (Tybor et al., 1975). Davila et al. (2007c) observed no significant difference between the foaming capacities of porcine plasma, serum, globulin and albumin at pH 4.5. However, a significant decrease in the foaming capacity of globulin was observed when the pH increased to 6.0 and 7.5. Plasma, serum and albumin showed very high foaming

capacities comparable to that of egg albumen at all pH values (pH 4.5 to 7.5). Foam stability varied with pH and the type of protein. All samples exhibited their lowest stability at pH 7.5 with no significant difference among them. At pH 4.5 to 6.0, the foam stability of plasma was slightly higher than that of serum and albumin and globulin at pH 6.0. The higher foam stability of plasma compared to serum at pH 4.5 and 6.0 was attributed to fibrinogen which is a more surface active protein (Davila et al., 2007c). Gomez-Juarez et al. (1999) studied the foaming properties of bovine globin isolated and decolourised by a process involving enzymatic hydrolysis of haemoglobin and oxidation by sodium hypochlorite. In contrast to the study reported by Vuono et al. (1979), globin showed good foaming capacity and stability, which increased as the pH increased from 2.0 to 10.0 (Gomez-Juarez et al., 1999), which may be attributed to differences in the method of isolation.

The foaming properties of lysozyme, BSA and β-casein were compared. The relative foaming abilities (time to reach half of the maximum foam volume) of lysozyme, BSA and β-casein were 30, 10 and 4 min. However, the maximum foam volume (foaming capacity) was 50% higher in the case of BSA than β-casein, while the lowest foaming capacity of about 20% that of BSA was observed for lysozyme. In terms of foam stability measured as half life of the foams, lysozyme was the best (>200 min), followed by BSA (32 min) and β-casein (15 min) in that order. The flexible structure of β-casein allows it to have higher foaming capacity, although the compact globule structure of lysozyme allows it to form a finer and stable foam (Kinsella, 1981). Prata and Sgarbieri (2008) compared the foaming capacity of egg white concentrate with that of BSA and bovine globulin. At 1% to 2% concentration, BSA and globulin were more effective foaming agents with much higher foaming capacity than egg white concentrate, while no difference was observed at 3% concentration (Prata and Sgarbieri, 2008).

The effect of deionisation (by ultrafiltration) and decationisation (by ion exchange) on the foaming properties of porcine plasma was studied (del Hoyo et al., 2008). Both deionisation and decationisation reduced the foaming capacity of plasma at pH 3.0 to 8.0, while some improvement was observed at pH 8.0 to 9.0. The highest foaming capacity was observed for the deionised plasma at pH 8.0 to 9.0. Good foam stability was observed in all cases, although the deionised plasma exhibited slightly less stability compared to the decationised and untreated samples (del Hoyo et al., 2008).

High pressure processing (400 MPa, 15 min) of the red cell blood cell fraction of porcine blood did not have a significant effect on the foaming capacity. On the other

hand, HPP improved the foam stability at pH 7.0, most probably through conformational changes (Toldra et al., 2008).

Ultrafiltration and freeze drying of plasma in the presence of 12% sucrose did not have significant impact on the foaming capacity of plasma powder at pH between 3.0 and 9.0. The highest foaming capacity was observed at pH 3.0 while the lowest was observed at pH 9.0. However, the process significantly improved the stability of the foam, which was attributed to the interaction between sucrose and the protein forming a complex with superficial activity that increased system viscosity and reduced foam drainage. Maximum foam stability occurred in both cases at pH 5.0 (near the isoelectric point) and minimum stability at pH 8.0 (Rodriguez-Furlan et al., 2010). On the other hand, mixing BSA with sucrose and trehalose (at 5:1 sugar to protein ratio), slightly reduced the foaming activity of BSA. However, the stability of the BSA foams were better in the presence of sucrose, while trehalose slightly reduced the foam stability. Both sugars had a slight protective effect on the foaming activity of BSA during air drying at 78˚C, while the opposite was observed with respect to foam stability (Murray and Hong-Jen, 1999), contrary to what was reported for plasma by Rodriguez-Furan et al. (2010). In general, sugars stabilise globular proteins against denaturation and increase protein diffusion to the air-water interface due to a more folded structure and decrease protein surface activity at steady state (Semenova et al., 2002).

Gelling properties

Gelling properties are important indicators of the quality of proteins for use in texture and mouth feel applications (Alvarez et al., 2009). Rheological parameters and water binding capacity are commonly used to characterise food gels.

Dehydrated blood plasma is used as a protein ingredient for its gelation properties, especially in meat derivative products. Generally, blood proteins are concentrated by either ultrafiltration or evaporation under reduced pressure. The resulting pH and ash concentration (mineral salts and sodium citrate) allow modification of the gelation properties of the products (Dailloux et al., 2002).

4.2.1.7 Plasma and its constituents

Plasma proteins form irreversible gels at temperatures above 55˚ C, depending on the pH and the protein composition. Plasma proteins are able to form a strong self-supporting gel at concentrations as low as 10%. Davila et al. (2007) reported that the gelation temperature of porcine plasma increased from 67.5 ˚C to 76.3 ˚C as the pH

increased from 4.5 to 7.5. A significant increase in the hardness of the gel was also observed with the increase in pH to 7.5. Likewise, the water holding capacity of the gel increased with increase in pH (Davila et al., 2007a). Similar effects of pH were reported in an earlier study on the gelling properties of ultrafiltrated bovine plasma. Gel strength increased with increasing pH from 3.0 to 7.0 for bovine plasma, with maximum strength at pH 7.0. Moreover, the strength of the plasma gel increased with increased heating up to 120 min. Higher gel strength was observed in the presence of 0.2 M NaCl, whereas increasing NaCl concentration above 0.2 M reduced the strength of the plasma gel. Plasma gels exhibited higher gel strength compared to that of egg albumen at pH 3.0 to 9.0. In contrast to plasma gels, prolonged heating above 100 minutes resulted in weaker egg albumen gels (Hickson et al., 1980). Alvarez et al. (2009) reported gelation temperatures of 71.4˚C, 56.8˚C, 44.8˚C and 56.9˚C for porcine plasma, fibrinogen, α- and β-globulin and γ-globulin respectively, at pH 6.0. The lowest gelation concentration for fibrinogen was 4%, while 2% was observed for the globulin fractions and 6% for whole plasma (Alvarez et al., 2009).

The gelation temperature of porcine serum (fibrinogen depleted, or a combination of albumin and globulin fractions) was reported to be 64.2˚C at pH 4.5, which increased to 72.5˚C at pH 6.0 and 74.4˚C at pH 7.5. The strength of serum gel increased with pH in the same range, while its water holding capacity remained constant. At pH 4.5 and 6.0, serum had higher gel strength than plasma, while the opposite was observed at pH 7.5. The water holding capacity was reported to be lower than that of plasma and albumin (Davila et al., 2007a). Adding an optimal amount of fibrinogen to porcine serum significantly improved the water holding capacity of the gel (Chen and Lin, 2002).

Matsudomi et al. (1991) studied the gelling properties of BSA. BSA formed a transparent and smooth gel with good water holding capacity during heating at temperatures higher than 70˚C. The firmness of the gel increased with heating at temperatures up to 95˚C and increased protein concentration. A minimum concentration of 4% was required for the formation of a self-supporting BSA gel at 90˚C. BSA gels had a maximum strength at pH 6.5 and as the pH increased to 8.0, the gel strength decreased significantly. NaCl at concentrations up to 0.4 M did not have a significant effect on gel strength at pH 8.0 (Matsudomi et al., 1991). A similar gelling temperature was reported by Davila et al. (2007) for porcine albumin at pH 7.5, although a significantly lower gelling temperature was observed at lower pH. The gelation temperature at pH 4.5 was 56.9˚C, which increased to 67.1°C at pH 6.0 and 73.7˚C at pH 7.5. The strength and the water holding capacity of the gel increased

with increase in pH to 7.5. Porcine albumin exhibited less firmness than porcine serum and whole plasma, while its water holding capacity was similar to that of plasma (Davila et al., 2007a). Alvarez et al. (2009) reported a similar gelation temperature of 67.7˚C for porcine albumin at pH 6.0, with 4% identified as the lowest concentration for the formation of a self-supporting gel (Alvarez et al., 2009).

The addition of α-lactalbumin at concentrations higher than 3% remarkably increased the gel strength of BSA without affecting its transparency, although α-lactalbumin did not form a gel by itself, reflecting the impact of protein to protein interaction, which is important in real food systems (Matsudomi et al., 1993). Compared to β-lactoglobulin, BSA gels were firmer at temperatures higher than 80˚C at pH higher than 6.5, although the gelling temperature of β-lactoglobulin was lower (70˚C) and it formed a stronger gel at temperatures between 70˚C and 80˚C. In contrast to BSA, the strength of β-lactoglobulin gels decreased significantly with increase in calcium concentration above 0.04 M (Matsudomi et al., 1991). Similarly Hegg (1982) reported that BSA showed good gelling properties during heating at 95˚C, better than that of β-lactaglobulin at a wide range of pH and salt concentration (pH 3.0 to 10.0, and 0 to 0.34 M NaCl) (Hegg, 1982).

Rodriguez-Furlan et al. (2010) studied the gelling properties of untreated and ultrafiltered plasma freeze dried with 12% sucrose as a stabiliser. The demineralised plasma powder formed a firm gel after heating for 30 min at 90˚C only at 12.5% concentration or higher at pH 3 to 6. Little or no gelation occurred at pH 7.0, 8.0 and 9.0. The highest gel strength was observed at the average pI of the plasma proteins (pH 5.0). Untreated plasma samples formed pasty gels which could not be fully characterised. Microstructural analysis of the two plasma samples revealed that untreated plasma, freeze-dried without sucrose, had a disrupted structure where the native structure of the proteins was significantly modified, while the demineralised plasma showed a uniform distribution of globular structure of proteins held together by the saccharide, explaining the difference in the gelation properties of the two samples (Rodriguez-Furlan et al., 2010). Similarly, Baier and McClements (2001) reported that sucrose increased the gelation temperature of 2% BSA solutions (pH 6.9, 100 mM NaCl) due to its protective effect against thermal denaturation. The strength of the gels that were formed after holding the samples for 15 min at 90 ˚C decreased with sucrose concentration because of the reduction in the proportion of irreversibly denatured protein. On the other hand, the strength of the gels that were formed after heating the samples for 120 min at the same temperature increased with increased sucrose concentration due to the higher proportion of denatured protein

and increased protein-protein interaction (Baier and McClements, 2001). Sugars stabilise globular proteins against denaturation and increase the strength of protein gels due to increased protein-protein interaction (Semenova et al., 2002).

Apart from sugars, interactions with polysaccharides have significant impact on the gelation properties of blood and other proteins. The overall effect depends on the properties of the proteins and the polysaccharides as well as environmental factors such as pH. Cai and Arntfield (1997) studied the effect of sodium alginate, pectin and methyl cellulose on the gelation properties of BSA. The addition of 1.5% of the anionic polysaccharides pectin and sodium alginate increased the gelation temperature of 10% BSA solution by 10˚C to 80˚C at pH 5.7, which was attributed to the formation of BSA-polysaccharide complexes due to ionic interaction. The addition of the same amount of the neutral polysaccharide methyl cellulose decreased the gelation temperature to 60˚C, which was assumed to be due to hydrophobic interactions between the polymers promoting increased network formation. Poor gelation properties were observed for the BSA-anionic polysaccharide systems at pH below the isoelectric point of BSA (pI=4.9), whereas the gelation properties improved above the pI of BSA. At the natural pH of the mixture (pH 4.6), pectin at 0.5% to 1.5% reduced the gel strength of BSA at all BSA concentrations investigated (3% to 10%). In contrast, sodium alginate at concentrations of 1% to 1.5% improved the strength of BSA gels at the natural pH of the mixture (pH 5.7) for BSA concentration from 5% to 10%. The difference between the two anionic polysaccharides was attributed to differences in complexation patterns at pH below and above the pI of the BSA. The BSA-methyl cellulose system showed good gelation properties at high BSA concentration regardless of the pH, increasing the gel strength of a 10% BSA gel by about 3% times at 1.5% concentration (Cai and Arntfield, 1997).

Processing of blood proteins can also influence their gelling properties. Pares et al. (2000) studied the effect of high pressure processing (400 MPa, 5 min, 5°C and 40˚C) on the strength and water binding capacity of heat-induced porcine plasma gels. They observed no significant effect of high pressure processing on the two parameters, although pressure had a significant effect on the conformation of the protein (Pares et al., 2000). Treatment of porcine plasma with microbial transglutaminase improved the texture and water-holding capacity of plasma gels at pH 5.5, which was attributed to intermolecular covalent cross-linking. The observed improvement was dependent on enzyme concentration as well as reaction time. At optimal conditions, about 30% increase in gel strength and about 3% reduction in water was released after gel centrifugation (Saguer et al., 2007).

4.2.1.8 Haemoglobin and globin

Salvador et al. (2009) investigated the gelling properties of haemoglobin. Gelation occurred on heating a 17% haemoglobin solution at 80˚C for 45 min at pH 4.5 to 7.5. The softest texture was for the pH 4.5 gel while the highest firmness was observed for the pH 7.0 gel. The water holding capacity was highest in the pH 4.5 gels (93%) decreasing to 46% at pH 5.5 and further decreasing to about 42% at pH 7.5. The gels formed at pH 4.5 were completely different from those at higher pH. They were not consistent, felt adhesive and more like viscous dough. The others were firmer and looked like a sponge (Salvador et al., 2009). Alvarez et al. (2009) studied the gelation properties of porcine haemoglobin. They observed that haemoglobin formed only a weak gel at pH 6.0 and 8% concentration, exhibiting poor gelling properties compared to other blood components (Alvarez et al., 2009) .

Bovine globin formed a gel on heating only within a narrow pH range between 5.6 and 6.0. However, salt reduced its ability to form a gel at the same pH range (Autio et al., 1984; Wismer-Pedersen, 1988; Autio et al., 1985), which limits its application as an ingredient in processed meat products. Autio et al. (1984) studied the effect of heating for 30 min at temperatures between 80 and 95˚C on the viscosity of a 5% globin solution. No significant increase in viscosity was observed at pH < 5.0 and pH > 6.0. In the pH range between 5.4 and 5.8, a significant increase in viscosity was observed during heating at 95˚C resulting in a firm gel. The freeze-dried gels showed very high water-binding capacity. The gelation temperature was about 60˚C, although higher temperature was required for the formation of stronger gels (Autio et al., 1984). The pH range for the gelation of bovine globin decreased with increased salt concentration to 4.2 to 4.4 at 0.12M NaCl. Significant reduction in the gel strength was also observed at the same salt concentration. No gel formation or increased viscosity was observed in the presence of 0.2 M NaCl, where the solubility was reduced to under 30%. However, combining globin with an equal amount of plasma improved its ability to form a gel in the presence of 0.2 M salt at pH 5.0 to 5.4. Plasma gels were firmer (about 4 times) than either globin or globin-plasma gels at pH 5.0 to 5.4 while the plasma-globin gels were slightly firmer at pH 5.6 to 6.0 in the presence of 0.2 M salt at 80˚C. The authors also compared the gel strength of porcine globin with that of bovine globin. Bovine globin formed gels with more than twice the gel strength compared to porcine globin, which was attributed to different amino acid sequence (Autio et al., 1985).

Water binding capacity

Water binding capacity is crucial when proteins are used as fat replacers since the replacement of fat by moisture is limited by the ability of the product to hold moisture (Miller, 1993). Most water binding capacity data on blood proteins are reported in relation to the gelation properties and the functionality of the proteins in food systems. Autio et al. (1984) studied the water binding capacity of spray-dried porcine globin isolated by the CMC adsorption method. The water binding capacity of globin was found to be much higher than soy protein, lactalbumin, and wheat gluten. Freeze-dried globin which has been isolated in the same way showed less water binding capacity than all the samples investigated, probably due to differences in porosity resulting from different drying techniques. The water binding capacity decreased with increase in ionic strength with about 80% reduction in the presence of 1 M NaCl (5.0 ml/g to about 0.9 ml/g). The water binding capacity remained almost the same in the pH range between 3.0 and 5.0. Further increase in pH towards the pI of globin resulted in significant reduction, with about 75% reduction at pH 7.0 (from about 5 ml/g to about 1.2 ml/g) (Autio et al., 1984).

Table 3 summarises the functional properties of the main blood constituents.

Many functionality studies specifically for bovine blood plasma use spray-dried, frozen, dried or freeze-dried plasma either as plasma, serum (combination of albumin and globulin fractions), protein isolates of plasma fractions and hydrolysates of plasma fractions. Table 4 outlines information found in the scientific literature related to fresh or ‘wet’ bovine blood plasma as a functional ingredient. A few studies on dried plasma have also been included. In some studies, the origin of the blood plasma (animal species) and / or the form of the plasma (fresh, dried, frozen, freeze-dried, etc) is not stated in the scientific paper, or abstract (where full papers are not accessible or in foreign languages) hence these papers have been included and noted.

Table 3 : Functional properties of the main blood fractions

Blood component	Solubility	Emulsifying properties	Foaming properties	Gelation properties	Reference
whole plasma	Very high protein solubility (70 -100%), limited effect of pH range, limited effect of NaCl	Very good, higher emulsion capacity than soy protein isolate	Very good, comparable to egg white, foaming capacity and stability slightly dependent on pH	Very good, firmer gels than egg albumen at pH 3.0 to 9.0, increased firmness with heating and up to 0.2 M NaCl	(Alvarez et al., 2009; Hickson et al., 1980; Vuono et al., 1979)
albumin	Very high protein solubility (70-100%), no effect of pH, limited effect of NaCl	Very good, superior emulsion activity at pH 6.5 compared to ovalbumen, β-casein etc, high ES better than egg albumen, casein, whey powder etc	Very good foaming capacity and stability, comparable to egg white, higher foaming capacity and stability than β-casein	Very good gelling properties, firm, smooth and transparent gels at >70 ˚C and up to 0.4 M NaCl, better than β-lactoglobulin	(Davila et al., 2007c; Hegg 1982; Kinsella, 1981; Matsudomi et al., 1991; Ramos-Clamont et al., 2003; Vuono et al., 1979; Waniska et al., 1981)
globulin	Good (40 -90%), increase with increase in pH, increase with increase in concentration of NaCl	Good, EC as high as soy protein isolate	Good foaming capacity and stability, dependent on pH	Good, forms gel at relatively lower temperature and protein concentration	(Alvarez et al., 2009; Ramos-Clamont et al., 2003; Vuono et al., 1979)
globin	10-90%, depending on pH and method of extraction, low at pH 4.0-7.0,significant reduction in the presence of NaCl	Good at pH ≤5.0, EC as high as casein, egg white etc at low pH significant reduction in the presence of NaCl, dependent on method of isolation	Very good foaming capacity, poor foam stability although dependent on the method of isolation	Not good, form gel in narrow pH range (5.4-5.8), 0.2 M NaCl inhibits gellation,	(Autio et al., 1984; Autio et al., 1985; Gomez-Juarez et al., 1999; Lima et al., 2010; Miyaguchi et al., 1992; Silva et al., 2003; Wismer-Pedersen, 1988)

EC: emulsifying capacity, ES: emulsion stability

Table 4: Functionality of blood plasma proteins

Source	Functionality	Country of study	Findings	Publication
Model systems				
Slaughterhouse blood (origin of plasma not stated) Separated fractions were Freeze -dried	Gelling	Spain / UK	Investigation by DSC, Raman spectroscopy of protein-protein interactions during heat induced gelation of plasma revealed that plasma gels are stronger than serum gels, due to differences in disulphide bonds resulting in non-reversible changes during heat induced gelation. Fibriniogen has the ability to minimise β-sheet formation of most globular proteins during gelation and may influence stronger gel formation of plasma. Raman spectroscopy showed that heat induced structural changes to plasma and serum may be influenced by pH.	(Davila et al., 2006, Davila et al., 2007b)
Bovine serum (form of plasma not stated)	Flavour, Gel strength	Japan	Charcoal treatment of serum proteins can remove off flavours and improve the gel strength of 8% bovine serum proteins after heating at 80°C for 15 min at pH 7 and NaCl concentration of between 75-150mM. The gel strength of the charcoal-treated serum could be increased by the addition of egg white, ovalbumin and glutathione.	(Chen and Hayakawa, 2001)
Bovine blood plasma (form of plasma not stated)	Gelling	Venezuala	pH 7 is the optimum for gel formation of bovine plasma	(Benitez et al., 1999)

Blood plasma (origin of plasma not stated) (form of plasma not stated)	Gel structure		Heating of blood plasma may cause local aggregation of proteins in gels resulting in a coarse structure where moisture is more easily lost. This may be accentuated by higher salt and protein concentrations and can be decreased by adjusting the pH away from the isoelectric point.	(Hermansson, 1983)

Source	Functionality	Country of study	Findings	Publication
Bovine blood plasma (form of plasma not stated)	Emulsification Hydrophobocity Solubility	Brazil	Bovine blood plasma was tested as an emulsifier and/ or fat replacer in food at pH 3 – 8. Maximum hydrophobocity was found to be pH 3.0, while maximum emulsifying activity index was at pH 7.0. Other functional properties including solubility and emulsion capacity were relatively constant across the pH range. Addition of NaCl (0.034mol/L) didn't affect emulsion stability but reduced solubility, hydrophobocity and emulsion capacity at pH 5 and 6 and increased emulsion activity index at pH5.	(Silva and Silvestre, 2003)
Bovine blood plasma (powder/solution)	Viscosity Solubility Protein structure	South Korea	Plasma protein powders and solutions were prepared from slaughter house blood and treated using gamma irradiation at 1-10kGy. Viscosity, solubility and secondary protein structure were not affected in irradiated powders, but were affected in plasma protein solutions causing aggregation of plasma proteins	(Lee et al., 2003)
Bovine Blood plasma	Gelling	Norway	Bovine serum albumin (BSA) appears to be the dominating gel-forming protein of bovine plasma. From differential scanning calorimetry (DSC) BSA was found to be 4°C-5°C more stable at pH 6-6.5 than at pH 7.8.Addition of 4% NaCl at pH 5.8 can also increase the stability of BSA by 4°C or by 10°C-11°C when free fatty acids are present at 9.6 moles/mole BSA at pH 5.8. Maximum gel strength was at pH 6.2 (7X that pH 7.8) and could be doubled by raising the temperature from 60°C to 74°C. Increasing the free fatty acid concentration (from 5mg/100mL) 10X can reduce gel strength by 30% at pH 5.8.	(Fretheim and Gumpen, 1978)

Bovine blood plasma (form of plasma not stated)	Heat stability Foaming capacity & stability Emulsification	USA	Blood plasma proteins were compared with egg white for heat stability, foaming capacity and stability and emulsification properties. All blood plasma proteins had lower denaturation temperatures than egg white, with gamma globulin the most heat stable and fibrinogen the most heat sensitive. Foaming capacity was similar to egg white but foam stability less and emulsification properties of blood plasma and component proteins better than egg white	(Raeker and Johnson, 1995)
Use in Products				
Blood plasma (origin of plasma not stated) Spray dried animal plasma (AP-820, from APC-Europe (Spain))	Gel strength Water holding capacity Fat emulsifying capacity	Spain	The functional properties of spray dried animal plasma (SDPA) were evaluated in isolated conditions (diluted in water) or in a petfood loaf product compared with other binding agents commonly used in pet food such as carageenan mix, wheat gluten, egg albumen or pork protein. SDPA was found to have high gel strength capacity above 90°C with a peak gel strength at 121^{o}C, while water holding capacity of the gel was found to be stable above 80°C. When compared with other binding agents, SDPA was found to be soluble, with better gelling properties than egg albumen, wheat gluten, and porcine protein but less than carrageenan. In a petfood loaf product, SDPA maintained a more compact texture and less exudation when substituted for other binding ingredients at the same level. Exudation losses for the 20g/kg SDPA formula were less than the 50g/kg wheat gluten formula with similar gel strength.	(Polo et al., 2005)
Bovine plasma (origin of plasma not stated) (form of plasma not stated)	Binding	Spain	10% w/w plasma can improve the stability (structure) of raw restructured bovine,meats where 0.75% transglutaminase is added, when tested by slicing at refrigeration temperature. This effect was not seen for porcine, or poultry meats.	(Marquez et al., 2008)

Bovine blood plasma solution (form of plasma not stated)	Yield	Venezuela	Cooked hams were formulated with bovine plasma and /or red cells as a partial replacement of the water content. Hams with plasma proteins at 3.6% and 5.4% gave better yields and at least 1.8% plasma protein was required to increase yield. Sensory evaluation of hams was regarded as acceptable.	(Rodas et al., 1998)
Bovine blood plasma (form of plasma not stated)	Formulation, Nutritional	Venezuela	Animal by-products including deboned poultry meat, bovine plasma and red cells were used to formulate a low cost high nutritional value meat product. The product was vapour cooked at 92°C to an internal temperature of 70°C. A product of 40% poultry meat, 20% plasma and 1.5% red cells was found to be acceptable No mention of human or animal product, what other ingredients were, or if blood was wet or dried.	(Benitez et al., 2000)
Bovine blood plasma (form of plasma not stated)	Emulsion stability, Yield, Protein content	Venezuela	Bovine plasma was used to replace part of the lean meat source of an emulsion-type product. Results showed the addition of plasma improved emulsion stability, yield and protein content, higher phenylalanine and valine content and had no affect on colour acceptability.	(Marquez et al., 1997)
Blood plasma (origin of plasma not stated) (form of plasma not stated)	Water binding, Yield	Germany	Addition of blood plasma during the manufacture of Bruehwurst sausages, improved water binding, increased yield without adversely affecting appearance or aroma, It also had economic benefits. No mention of plasma origin.	(Linke et al., 1983)

Blood plasma concentrate (origin of plasma not stated) (form of plasma not stated)	Emulsification, Gelling, Formulation, Sensory	Germany	Blood plasma concentrate (BPC) (10.5% protein, pH 7.68) produced by ultrafiltration was used to replace ice at 2.55-9.0% or meat (lean pork) at 2.5%- 11.25% in frankfurter production. Results showed increasing pH with increasing levels of blood protein concentrate added. When replacing ice, jelly separation was reduced, however at heat treatments of 110°C for 45 min, the amount of jelly separation was dependent on BPC level added. Heat treatments of 100°C for 35min resulted in a firmer product. When used to replace lean meat, BPC reduced jelly separation but had a different effect to diphosphate. A reduction in firmness was noted in all batches. Addition of BPC above 6.75% resulted in sensory changes.	(Murmann and Wenzel, 1986)
Blood plasma (origin of plasma not stated) (form of plasma not stated)	Viscosity, Texture	Japan	Plasma protein that had been alkali –treated to pH 11 - 11.5 showed considerable increases in viscosity and good spinning characteristics at pH 11-13.	(Suzuki and Miura, 1981)
Blood plasma (origin of plasma not stated) (form of plasma not stated)	Water binding capacity, Formulation, Nutritional,	USSR	Liquid blood plasma and a blood plasma protein were compared for use in the replacement of the lard content of raw smoked sausages. Both ingredients resulted in a product of increased moisture and protein content which remains stable during storage. The water binding capacity of sausages made with blood plasma was higher than those made with a blood plasma protein.	(Zharinov et al., 1981)
Blood plasma (origin of plasma not stated) (form of plasma not stated)	Colour stability	Poland	The influence of 10% addition of blood plasma on the colour of canned ham stored at refrigeration temperatures for more than a year showed no influence on colour parameters. It was also found that during the first 3 months of storage, blood plasma addition resulted in hams of increased colour stability when exposed to white light.	(Dolatowski, 1988)

Blood plasma (origin of plasma not stated) (form of plasma not stated)	Tenderness Microbiological quality	Poland	The addition of 10% blood plasma to cured, canned beef stored at refrigeration temperatures for a year resulted in increased tenderness of the beef product and had no effect on microbiological quality.	Dolatokowski, 1988,
Synergies of other ingredients with plasma proteins				
Interactions	Enzymic starch degrading ability of meat and plasma	Sweden	Starch degradation was observed when blood plasma was combined with potato flour, heated to 60°C then stored. When used in sausages, maximum starch degradation was found when samples were heated to 70°C.. After 7 days storage at 60°C, hydrolysis of about one third of the starch to glucose was observed. It is likely that this may influence textural quality, in particular the water binding capacity.	(Skrede, 1983)

Shelf-life				
Bovine blood plasma	Shelf life		Effect of temperature-time on plasma bacterial count was tested of blood from bags and a storage tank. Aerobic plate count (APC) and total coliforms (TC) higher for tank than bags. Refrig storage 4 days @5C: - counts increased during storage. Frozen @ 15°C for 7 days; - unchanged APC and decreased TC Heat @ 45°C for 5 min; - unchanged APC and decreased TC Heat @ 45°C for 10min; - decreased APC and TC Heat @ 48°C for 5 min; - decreased APC and TC, caused turbidity of plasma Heat @ 60°C at 33mL/min using a milkotester; - decreased APC and TC. S Aureus and Salmonella not present.	(Medina et al., 1997)

4.3 Current applications of blood in the global food industry for functionality

Blood proteins make up the largest part of the meat proteins market by volume. Products are supplied mainly as powders and in their frozen form. Blood products typically contain ~70% protein, and blood components such as plasma, haemoglobin, immunoglobin and globin are used in a number of food applications.

Plasma is often used in sausages to help bind and emulsify meat, haemoglobin is used in pet food and to colour black pudding, immunoglobin can be used as an emulsifier in meat products, while globin is used in mincemeat and hamburgers, where it improves textural properties and to some extent helps bind ingredients together. Blood proteins such as immunoglobulin are also used in the pharmaceutical industry in the preparation of antibodies and other immunoassay products (Frost & Sullivan, 2005). The pharmaceutical industry also creates isolates such as creatine from blood proteins, to be used in nutraceutical applications. Although the demand from the pharmaceutical industry is increasing, it only constitutes a small fraction of the blood protein market.

4.4 Application of blood and blood components as food ingredients and comparison to other functional protein ingredients

Searches were carried out on both Derwent Innovations Index and Food Science and Technology Abstracts for applications for wet blood plasma proteins that might help add detail to its functionality. These searches mostly produced applications from food cultures that find inclusion of blood in food products acceptable, i.e. they were mostly from Asian or eastern European countries and typically foods such as blood sausages and other smallgoods or meat products in general. Unfortunately, these mostly either did not use plasma proteins, or were applications that did not significantly add degrees of detail on the functionality of the blood products that were likely to be helpful.

Whole blood and blood-derived proteins have many potential applications as ingredients in food products. As with other proteins, blood-derived proteins have good nutritional and functional properties that can be utilised in conventional and semi-conventional food products (Vuono et al., 1979).

Blood is a good source of readily absorbable iron (Duarte et al., 1999) which can be used to produce nutritional supplements. Heat processing of blood tends to decrease the bioavailability of iron, by converting haem iron (bioavailable) to non-haem iron (not bioavailable). It has been shown that heating pig blood to 55°C and above significantly increased the non-haem iron content, while decreasing the haem iron content. Above this temperature, there was a linear relationship between temperature of heating and conversion of haem iron to non-haem iron (Wang and Lin, 1994). Additional studies have also shown that when RBC were heated, the non-haem iron concentration increased from 3.4% to 10.2% when heated slowly, and to 6.9% when heated rapidly (King et al., 1990). Thus, rapid heating will decrease the bioavailability of iron from RBC.

It would appear that the absorption of iron is improved when it is fed in conjunction with meat constituents (Etcheverry et al., 2006). Work on rats has demonstrated that haem iron in a digested muscle extract was more readily absorbed than haem iron in an aqueous extract, suggesting that it is not the haemoproteins themselves that are responsible for the high bioavailability of iron from meat products, but more due to the products of the digestive process in a meat-related substrate (Hazell et al., 1978). It has been demonstrated in a model digestion system, that meat, RBC and haemoglobin supported the reduction of non-haem ferric iron (FeIII) to the ferrous form (FeII), which is more bioavailable, which could account for the observed increased bioavailability of iron in meat-based diets (Kapsokefalou and Miller, 1991).

Although blood has low methionine and isoleucine contents, it is a rich source of other essential amino acids such as lysine and can be used for fortification of processed cereal products (Wismer-Pedersen, 1988). Bates et al. (1974) added whole blood to yeast-raised bread. The baking properties were not affected, although the loaf volume and the crumb texture (became coarser) were significantly affected (Bates et al., 1974).

Bovine blood has been fractionated into blood plasma protein concentrate, red blood cell concentrate, globin isolate, and a carboxymethylcellulose-heme iron (CMC-heme) complex. The amino acid content in plasma protein concentrate has been shown to be well balanced and produced net protein utilization and a net protein ratio equivalent to 95% that of casein. Globin isolate (similar to 91% protein) is deficient in isoleucine and S-containing amino acids and was unable to support rat growth at a 10% concentration in the diet. Red blood cell concentrate and the isolated CMC-heme complex were good sources of bioavailable iron. Iron availabilities for CMC-heme and whole blood cell concentrates were 64 and 70% of ferrous sulfate respectively (Duarte et al., 1999).

As discussed in Section 4.2, blood plasma proteins have comparable functional attributes to traditionally used protein ingredients (Table 3) such as egg white and can be used as an alternative. Several studies in the literature have been reported on the application of blood plasma and its constituents such as albumin as functional ingredients in processed meat, bakery and pastry products.

One to 2% added dried plasma significantly improved the shrinkage of sausage during cooking with an equivalent effect to that of 3% soya isolate. Replacing up to 8% of the lean meat with plasma did not have a significant effect on the texture, flavour and colour of sausage and the shrinkage was also significantly reduced, with the highest reduction occurring when 8% of the lean meat was replaced with 2% blood plasma (Ranken, 1977). Lu and Chen (1999) compared the binding strength of plasma powder from broiler, lamb, porcine and bovine sources with egg white and other binding agents for binding restructured muscle product composed of chicken breast, pork loin and pig backfat. They compared the efficacy of the proteins for muscle to muscle, muscle to fat and fat to fat binding. Egg white powder gave the strongest muscle to muscle binding, followed by porcine plasma powder, bovine plasma powder, lamb plasma powder, chicken breast powder, broiler plasma powder, soy protein breast meat powder, wheat gluten, gelatine, whole egg powder and sodium alginate, in that order. The plasma powders performed much better than soy protein and the other binding agents. Similar results were obtained with respect

to muscle to fat and fat to fat binding, with egg white powder performing best followed by the plasma powders from different sources (Lu and Chen, 1999).

del Rio de Reys et al. (1980) evaluated the effect of fortifying wheat flour with chicken plasma on the quality of bread. The addition of dialysed plasma by as much as 7.5% resulted in a significant increase in loaf volume. However, the addition of plasma greater than 2.5% negatively affected crumb colour, bread aroma and taste, reducing its acceptability (del Rio de Reys et al., 1980). Johnson et al. (1979) evaluated the use of bovine plasma in cakes as a replacement for egg. The cakes made with plasma had at least an equivalent volume to the control cake baked with egg white, with good profile and crumb texture. In addition, the crust colours of the plasma cakes were more golden (Johnson et al., 1979). Raeker and Johnson (1995) compared the cake-baking properties of bovine plasma proteins with egg white. Egg white performed slightly better than whole plasma. It produced slightly larger volume, significantly more crowned profile and finer crumb structure. Among the plasma proteins, fibrinogen produced the smallest cake volume. Compared to whole plasma, γ-globulin had better cake-baking properties whereas albumin was inferior. Separation of fibrinogen from plasma significantly improved the cake-baking properties of plasma (Raeker and Johnson, 1995).

Bovine plasma contains protease inhibitors and can be used to inhibit softening of Pacific whiting surimi gels (Morrissey et al., 1993; Seymour et al., 1997; Kang and Lanier 1999). Kang and Lanier (1999) compared the effect of bovine plasma with three specific cysteine protease inhibitors on the Pacific whiting surimi gel strength. They observed a significantly higher effect of blood plasma proteins than the cysteine protease inhibitors and concluded that the gel strengthening effect of plasma can not only be attributed to the protease inhibitors but also to the other proteins present in plasma contributing to the gel strength (Kang and Lanier, 1999). Bovine plasma has also been found to be a more effective inhibitor of proteolysis in surimi gels compared to egg white and potato (Morrissey et al., 1993). Porcine plasma proteins were also reported to be effective inhibitors of fish proteinases, although the proteins interfered with the gelation of actomyosin resulting in a lower gel modulus (Visessanguan et al., 2000).

Globin has good foaming and emulsifying properties, although it can form heat-set gels only in a narrow pH range and salt reduces its ability to form a gel. However, the properties of globin can be improved by combining it with an equal proportion of plasma (Autio et al., 1985). An acceptable product was obtained by replacing 10% of meat in Bologna sausage with an equal proportion of plasma and globin (Calderoni

and Ockermann, 1982). Up to 25% of lean meat in model sausages could be replaced by an equal proportion of plasma and globin proteins (Wismer-Pedersen, 1988). Up to 50% of the egg in biscuit production could be replaced by decolourised blood made by combining globin with plasma without significant effect on crumb porosity and compressibility (Mitsyak 1975) as reported in (Wismer-Pedersen, 1988). Ribeiro Viana et al. (2004) studied the effect of the replacement of fat in ham pâté by bovine plasma, globin and plasma and globin in equal proportion. The replacement of fat with the blood proteins resulted in some reduction in the water holding capacity of the raw batter. The highest reduction was observed for plasma followed by the plasma-globin mixture. Globin improved the raw batter stability, while some reduction in stability was observed in the case of the plasma and plasma-globin pâté. This was attributed to the good emulsifying properties of globin stabilising the emulsion (Ribeiro Viana et al., 2004).

From the results of the patent searches, there are a number of patents where whole blood, whole plasma and its components, and globin are used as ingredients in food products. Many of the meat products incorporated whole blood or blood plasma (both wet and dried) or protein products from haemolysed blood and the inclusion of blood plasma was often to exploit its water binding capacity. Isolated proteins such as fibrinogen were also a feature of some of the older patents. More recent applications generally used hydrolysed proteins. As many of these are translated it can sometimes be difficult to have confidence in the accuracy of the detail and for some foreign materials it was hard to identify why proteins sourced from blood had specifically been shown in the IPC Codes. Searches for general food products sometimes located items with blanket claims for all proteins or for both plasma and its protein components. Tables 5, 6 and 7 summarise this material.

Table 5: Applications of blood constituents as food ingredients based on patent literature

Blood component	Functionality/use	Products/product category	Comments	Reference	Coverage
Blood	Improved nutrition and texture	Noodle	Spices used to hide the blood smell	(Li, 2010) (Wang, 2007)	Chinese
	Nutritional supplement, therapeutic and prophylactic	Breakfast product containing dried blood		(Faivishevskii and Lisina, 1997)	Russian
	Improve nutrition, water and fat binding capacity, and structure of the dough	Bread	Dried decolourised blood used	(Karnaushenko et al., 1992)	Russian
	Foaming, emulsifying, nutrition	Chocolate	Filtered blood partially replacing milk	(Faracchio, 1984)	German
	Impart meat like taste and flavour, improved consistency	Meat analogue		(Belyaev et al., 1992)	Russian
Blood proteins	Gelling, emulsifying	Emulsion type foods: Mayonnaise, salad dressings, dips, sauces, sour cream, cultured creams		(Ye and Taylor, 2008)	World
Fibrinogen	Binding/ (adhesion) Improving consistency	Meat, fish and vegetables		(Roodink et al., 2003)	World

Blood component	Functionality/use	Products/product category	Comments	Reference	Coverage
		Semisolid foods e.g. Dairy products			
Plasma	Gelling and nutritional fortification	Fruit jelly		(Antipova and Kulpina, 1998)	Russian
	Emulsifier	Food emulsions such as mayonnaise, soup, salad dressings		(Ogasawara et al., 1999)	European
	Emulsifying, gelling, meat extender, binder	Ham and sausage, other processed meat products		(Taic, 1990) (Karasek et al., 1984)	Japanese World
				(Leger, 1985)	Canadian
	Improve texture	Noodle		(Taic, 1989)	Japanese
	Caviar analogue	Food		(Muschiolik et al., 1984) (Thota and Rebstock, 1996)	France USA
	Edible coating	Meat, fish, seafood, poultry as an alternative to batter		(Bueker et al., 2010)	
	Edible casing	sausage	As an alternative to egg protein etc		German
	Emulsifying agent			(Roberts, 2009)	UK
		Wafer, crisp	As an alternative		

Blood component	Functionality/use	Products/product category	Comments	Reference	Coverage
	Improve water binding and nutritional quality	Cured meat products	to whey protein etc Part of the brine soln	(Ilina et al., 2008)	Russian
Serum	Emulsifying agent	Emulsion foods: sauces, meat products		(Kanf, 1989)	Japanese
Bovine serum albumin	Fat substitute, emulsifier	Fat containing foods, dressings, spreads, soft cheese etc	As an alternative to egg white, whey etc, the protein is microparticulated	(Bringe, 1993) (Cox and Norton, 2010)	World World
	Microencapsulation delivery system for lipophilic nutrients and flavorants, phytochemicals	Beverages	As an alternative to whey protein etc	(Crouse et al., 2010; Given and Given, 2009; Given et al., 2009)	World
	Protease inhibitor, gelling, texture improvement	Surimi	Pig albumin and pig serum also included	(Choi et al., 2008)	Korean
	Gelling	pH responsive hydrolgel as meal replacer for weight control		(Adams et al., 2006)	World
	Foaming	Beer-like drink	As an alternative to egg white, soy protein etc	(Oono and Ono, 2004)	World

Blood component	Functionality/use	Products/product category	Comments	Reference	Coverage
	Foaming, emulsifying, Reduced spattering during frying (for margarine), improved texture Emulsifier, nutrition	Emulsion type foods-margarine, mayonnaise, ice cream etc Infant formula	Any serum albumin as an alternative to egg white etc As an alternative to whey protein	(Benjamins et al., 2000; Benjamins et al., 2002) (Braun et al., 2001)	World World
Red blood cells	Fe and protein fortification, emulsifier	Cream, paste, glaze etc	Flavourings used to hide the taste	(Antipova and Rozhkov, 1999)	Russian
Haemoglobin	Colorant	Food stuff Sausage		(Polo Pozo et al., 2003) (Antipova et al., 2007)	World
Globin	Foaming agent, emulsifier, meat extender	Sausage, cornered beef, other processed meat products		(Wismerpede et al., 1989) (Halasz et al., 1984) (Luijerink, 1982)	World World USA
Haem	Fe supplement, fortification			(Wismerpede et al., 1989) (Luijerink, 1982)	World USA

Table 6: Meat and meat-like product applications for bovine blood plasma proteins

Inventor Assignee	Patent Number	Title	Details
Muschiolik, G. Schmandke, H Wagenknech, W. Runge, G Peterson, J. INST HOCHSEEFISCHEREI AKAD WISSENSCHAFTEN DDR	FR2553262-A1; DE3432309-A; FR2553262-A; GB2148090-A; NL8402800-A; DD219378-A; JP60156371-A; HU38815-T; GB2148090-B	Granular protein prod. resembling caviar - prepd. by heat coagulating soln. of plasma of controlled protein concn.	Prodn. of granular protein shaped articles with sensory properties resembling caviar uses as starting material an aq. soln. contg. heat-coagulatable proteins and additives… the new feature is that the soln. is based on stabilised, liq. animal plasma of protein content 7-10.5 wt.% in which the protein content has been increased by centrifugation or opt. by adding dried proteins, esp. spray-dried or freeze-dried cattle blood plasma…By adjusting the protein concn., prods. of uniform quality are obtained regardless of the presence of anticoagulants or of the method of plasma recovery.
Zharinov, A.I Basilio, P. K Marta, T. G MOSCOW MEAT DAIRY INST	SU888899, 1981	Prodn. of smoked or part-smoked sausage - including adding mixt. of blood plasma and plasma protein sausage meat mixt.	Baked or boiled smoked or part-smoked sausage is prepd. by making a sausage meat to which is added a 1:3 to 3:1 mixt. of ppted. blood plasma protein and liq. blood plasma, the mixt. being added in an amt. 15-30wt.% on wt. of initial sausage meat ingredients. The sausage is then shaped and heat treated. The sausage has good structuring properties and good organoleptic properties; it has a low final moisture content and good storage life.(DDI Abstract)
NIPPI COLLAGEN KOGYO KK	JP2004131741-A 2004	Adhesive composition for sticking natural intestines for use in sausage casings, contains adhesive agent e.g. polysaccharide and/or animal protein, for	An adhesive composition contains adhesive agent for connecting at least 2 natural intestines such that lumina is connected mutually. The adhesive agent is polysaccharide, vegetable albumin, animal protein, collagen, gelatin, egg protein, lactoprotein, blood protein…fr

Inventor Assignee	Patent Number	Title	Details
		connecting natural intestines such that lumina is connected mutually.	sticking/adhering natural intestines for use in sausage casings.
NIPPON OILS & FATS CO LTD (NIOF)	JP4320662-A 1992	Processed meat prepn. with improved taste, flavour and appearance - by emulsifying edible oil and water using plasma protein, then injecting in to meat.	20-70 wt% of edible oil and 30-80 wt% of water are emulsified by use of plasma protein. The obtd. emulsified soln. is injected to meat. USE: Taste, flavour and appearance of meat contg. low fat are improved. Juicy and marbled state meat is effectively obtd..
CHIBA SEIFUN KK	P1060354-A; 1989 JP2612171-B2 1997	Compsn. for improving sausage quality - contg. heat solidifying protein and calcium cpd..	Compsn. contains heat solidifying protein (e.g. wheat protein, soy protein, egg white, blood protein and lactic protein) and Ca cpd…Compsn. improves quality (e.g. retention of water, emulsification, elasticity) of sausage without adding phosphates.
FUJI SHOJI KK (	JP63012269-A; 1988 JP91073262-B 1991	Additive compsn. for improving quality of paste prod. - contains carrageenan powder, natural gum, glyceride powder and plant or animal protein powder.	Additive compsn. comprises 1-50 wt% of carrageenan powder; one or more natural gum…,1-99 wt.% of glycerides powder selected from corn syrup, dextrin, maltose, etc.; and 2-80 wt.% of one or more plant or animal protein powder selected from blood protein, collagen, gelatin, whey protein, wheat protein, soy protein, and fish protein. The additive improves emulsification, water retention, elasticity, and adhesiveness in mfr. of sausage or ground fish meat.
Rogov, I.A. Zhuravskay, N. K Roslova, A. P MOSCOW MEAT DAIRY INST	SU959728-A 1982	Prodn. of cooked meat sausage - from minced meats, salts, skim milk or whey, blood plasma protein, sodium pyrophosphate or polyvinyl-alcohol and stabilised blood.	Cooked meat sausage is made from sausage meat contg. milk and blood plasma proteins the mix is shaped and cooked. A better yield is secured of a product with a firmer structurised gel, if the milk protein consists of skim milk or whey at pH 4.8-5.8. The plasma proteins are in liquid state (pH 6.8-8.1). Plasma and skim milk

Inventor Assignee	Patent Number	Title	Details
			are used in the ratio 3:1-1:4. 25-50% of this mixture is added to the sausage meat.…
UENO PHARM CO LTD	JP58098061-A; 1983 US4464404-A 1984	Increasing elasticity and chewability of fish meat cake - by adding blood plasma protein to minced fish meat.	In the prodn. of fish meat cake, (1) blood plasma protein is added to (2) minced fish meat. Addn. of (1) improves gel strength of moulded fish paste. (1) consists mainly of albumin and globulin, and is obtd. pref. from cattle, pigs or poultry. Amt. added is 0.1-3%, pref. 0.2-2% (dry w/w). It may be used as a dry powder or liq. Other protein, e.g. egg white, casein, lactoalbumin, gluten, etc. may be used together….
Lynch, C. J Lin, C. F Melachouri, N. (TAUFFER CHEM CO (NUTRISEARCH CO)	EP46639-A; 1981 US4348420-A 1982	Binders for comminuted meats contg. whey derivs. - with hydrocolloid to increase binding efficiency and chopping resistance.	Comminuted meat prods. are bound using a compsn. contg. 50-99.5% of a water soluble protein-contg. mat'l and 0.5- 50% of a hydrocolloid. The protein esp. includes a whey deriv. The binder comprises ... The protein can be derived from animal or vegetable sources, and may be e.g. milk, alkali metal caseinate, blood protein, solubilised meat, soy protein etc. certain whey prods. available in large quantities.
Huang, Y. Yu, W Shang, Y. Wang, Y. Feng, J. UNIV CHINA CENT NATIONALITIES	CN1860930-A 2006	Simple effective method for producing recombination meat.	Method for preparing a big recombinant meat block from the small meat blocks features that the cheap blood plasma of animal is used to prepare adhesive and the adhesive is used to adhere the small meat blocks together to become a big meat block. It has high adhesion.
Lipatov, N.N. Rogov, I. A Chagarovskii, A.P (ODESS FOOD IND TECH MOSC MEAT	SU1745182-A1 1992	Protein meat substitute prodn. - by ultrafiltration-concn. of blood plasma, addn. of milled fat tissue, and treatment with aq. soln.	The method is based on ultra-filtration concn. of blood plasma, aeration, introduction of milled fat tissue, and destabilisation with a soln. of calcium chloride to pCa equal to 3.15 - 3.25. In prod. of analogue of beef, the ultrafiltration concn. of

Inventor Assignee	Patent Number	Title	Details
DAIRY INST)		of calcium chloride.	plasma is conducted until the content of solids in plasma reaches 22-24% and milled beef fat is used as the fat tissue, added in amt. 4-6%. In the prodn. of pork analogue, ultrafiltration concn. is carried out to the content of solids in plasma equal to 17-19% and pig back-bone or hip-bone fat is used as the fat tissue, added in amt. of 25-45 medium, at 18 deg.C, until its structure is fixed. Tests show that obtd. protein beef analogue, made from 94-96 kg of blood plasma of protein content 17-18% and fat content 1%, has moisture content 75.44-78.68%, protein content 15.9-17.125% and fat content 4.317-5.43%. Pork analogue, made of 55-75 kg plasma of protein content 17-17.5%, and fat content 1% has moisture content 47.96-62.33%, protein content 9.98-13.37% and fat content 23.2-40.96%. for use in dietary meat prods with compsn. resembling natural meat prods.
Ilina, N. M Ilin, V. E Popova, N. A. Durakova, L.V (ORON TECHN ACAD)	RU2315482-C1 2008	Meat salting composition for obtaining of baked ham products.	Meat salting composition contains edible salt, sugar, phosphate, carrageenan, sodium nitrite, milk whey, animal protein of blood plasma, used as protein enricher for increasing biological value of product, and erythorbate for fixation of product color, said components being used in predetermined ratio. For use in meat industry, in particular, means for obtaining of baked ham products. Increased nutritive value of product owing to utilization of brine based on milk whey, and increased yield of ready product.
Koenig, M. Auf, D. H D Bueker, G.	S2007190210-A1; EP1820404-A1;	Single- or multilayer food casing used as outer casing, e.g. for sausage products,	Single or multilayer food casing comprises a carrier based on at least one water-insoluble, thermoplastic polymer (e.g. PVC), on a polymer

Inventor Assignee	Patent Number	Title	Details
Lunow, M. Wolf, H. Auf, D. E. R. Heide D Wolf, H. P Wolf, P. (ALLE GMBH)	DE102006027493-A1	comprises carrier based on thermoplastic polymer (e.g. PVC), and coating comprising binder (e.g. casein) and fine to coarse foods as additives.	of animal or vegetable origin or on regenerated or precipitated cellulose, and a coating comprising a binder (e.g. casein) and at least one additive which is transferable to the filling… The food casing is simple to produce. Claim included for: (1) a process for producing a food casing comprising coating a tubular or web-form carrier material with a binder, applying an additive, optionally coating the carrier-material with additional binder, and drying the coated carrier material; and (2) a method of transferring additives to foods comprising filling a food casing with food….The protein is whey protein, egg white protein or blood plasma…
Faivishevskii, M.L Krylova, V. B Grebenshchikova, T. Yu MEAT IND RES INST FAIVISHEVSKII M L	RU2160023-C2 2000	Meat product manufacture.	Method involves crushing raw meat material; preparing meat farce…with protein-fatty composition being used as viscosity and plasticity reducing component. Protein-fatty composition contains modified wheat flour, edible blood or blood elements or plasma or blood and water whey, and raw beef or pork fat.
ITO HAM EIYOSHOKUHI	JP60145069-A; 1985 JP87001703-B 1987	Sausage prepd. from conc. blood plasma - previously subjected to ultrafiltration to concn. rate above 2.	Method comprises (a) concentrating blood plasma through ultrafiltration to a concn. rate above 2; and (b) prepg. sausage using the concd. plasma as subsidiary material. Ultrafiltration can be practiced using an ultrafiltration membrane of fractionating mol. wt. below 200000. By increasing the concn. rate, the gelling strength of concd. plasma can be increased and for sausage use, concd. plasma of concn. rate above 2 is pref. used. The concd. plasma of higher concn. rate can intensify the elasticity of obtd. sausage…The blood of

Inventor Assignee	Patent Number	Title	Details
			slaughtered animals contains protein of good quality and it known as liq. meat. Its protein content is ca. 7-8% and its use has been restricted. By ultrafiltration, blood plasma can be concd. to the same protein content as meat easily without deterioration of its quality. The concd. plasma can be used for prepg. sausages as a substitute for meat.
NIPPON KAYAKU KK	JP58111661-A; 1985 JP90048226-B 1987	Processing of animal or fish meat food - involves addn. of water-soluble peptide and blood plasma protein, to provide gel intensification effect.	The process involves adding (a) a cold water-soluble peptide which is prepd. by hydrolysing animal protein and has av. mol. wt. 10000-20000 and, if necessary, (b) blood plasma protein to the food. Animal protein (e.g. gelatin, blood powder, etc.) has been used for intensifying the jelly strength of processed animal or fish meat food (e.g. ham, sausage, boiled fish paste, etc.) but being insoluble in cold water it has been inconvenient to use. The described animal protein-hydrolysed peptide having the specified mol. wt. is soluble in cold water and has excellent intensifying effect on jelly strength. By using it in combination with blood plasma protein its gel-intensifying effect can be further increased. Pref. collagen-type peptide is used in amt. 0.1-25 w/w%, pref. 5-10 w/w%, on objective food. Blood plasma protein is used 0.1-5 w/w%, pref. 0.3-2 w/w% on food.
TAIYO KAGAKU KK	JP6090710-A; 1994JP3072357-B2 2000	Prepn. of meat food e.g. ham, bacon, sausage, without excessive amt. of phosphate - using thrombin, and opt. plasma protein, fibrinogen concn. plasma,	Thrombin is used in prepn. of meat prod. produced by salt soaking process. Plasma protein or fibrinogen concn. plasma may be also used. Fibrinogen or plasma transglutaminase may be also co-used…Processed meat such as ham, bacon and sausage of high quality having

Inventor Assignee	Patent Number	Title	Details
		fibrinogen, or plasma trans:glutaminase, in prodn. of meat by salt soaking process	improved water retention property and elasticity is obtd. Excessive intake of phosphate is prevented.

Table 7: Non-meat applications for bovine blood plasma proteins

Inventor Assignee	Patent Number	Title	Details
Van Hulst M W (BIMAJA HOLDING)	WO2009007126-A1 2009	Preparation of vegetable chips or French fries involves preparing a mixture comprising a vegetable source or fruit source, a protein source, water and a thickening agent and molding the mixture prepared into chips or French fries.	The protein source 2-30wt % of the mixture (pref 4-15) is chosen form meat protein, blood protein…
Ye, A.	WO2008130251-A1 2008	Preparing a gel, comprises mixing oil or fat with an aqueous medium by homogenization to form an oil-in-water emulsion, and heating the mixture to form an emulsion gel.	The proteins are soy proteins, whey proteins (preferred, myofibrillar (skeletal or meat\ proteins, egg proteins or blood proteins….
Ye, A, Taylor,S. M.	WO2008130252-A1 2008	Viscoelastic fluid preparation used in/as food, e.g. mayonnaise comprises heating homogenized emulsion, allowing heat-treated emulsion to form a gel by adding salt or acidification, and subjecting gel to shearing	…the protein comprises whey proteins, soy proteins, myofibrillar (skeletal or meat) proteins, egg proteins or blood proteins, preferably soy or whey proteins…
Ye, A Taylor, S.M	WO2008130253-A1	Method to form gel involves mixing oil/fat and aqueous medium to form	The protein comprises whey proteins, soy proteins, myofibrillar (skeletal or meat) proteins, egg proteins or blood proteins, preferably soy or whey proteins

Inventor Assignee	Patent Number	Title	Details
		oil-in-water emulsion of heat-settable protein and oil/fat; homogenizing; heating to specific temperature to denature protein without allowing gel to form and adding salt.	
TAIYO KAGAKU KK	JP2004215543-A 2004	Noodle quality improvement agent for improving quality of Japanese noodles, Chinese-style noodles, sum buckwheat noodles, somen noodles, cold noodle and rice vermicelli, contains polyoxyethylene sorbitan fatty acid ester.	Noodle quality improvemtn agent further contains egg white powder yolk powder whole egg powder, casein, casein sodium, lactoprotein collagen gelatine, plasma protein…..
TAIYO KAGAKU KK	JP2004147576-A 2004	Agent for improving the quality of Chinese noodles, comprises calcium alginate as active ingredient.	Noodle quality improvement agent further contains egg white powder yolk powder whole egg powder, casein, casein sodium , lactoprotein collagen gelatine , plasma protein…..
TAIYO KAGAKU KK)	JP2004024155-A 2004	Agent for improving quality of noodles such as Japanese noodles, contains fine cellulose-containing composite which consists of specified amount fine cellulose, locust bean gum, xanthan gum and hydrophilic substances.	The fine cellulose composite contains egg white , egg yolk, egg protein , whey protein casein plasma protein
TAIYO KAGAKU KK	JP3098544-A; JP2896373-B2	Prepn. of noodles - from a basic dough contg. blood	A new sort of noodles with specific quality can be prepd. from a dough contg. wheat flour etc. as major components

Inventor Assignee	Patent Number	Title	Details
		plasma creating a better texture.	and a purified blood plasma of killed animals or its dry powder. Gives better texture to boiled noodles.
KANEKA CORP	P2002330702-A 2002	Filling material for use in confectionary, to prevent bumping of essential components during heating and baking process, comprises preset amount of protein, starch and emulsifier.	A claim is included for preparation of filling material, which involves adding filling material such as (in weight %) protein (0.1-15), starch (0.1-15) and emulsifier (0.02-1) to fats and oil composition (2-20). The protein is egg protein wheat protein, soybean protein, blood protein …..
Ogasawara, M., Yamamoto, K., Watanabe, M. Masashi, O Kazuhiro, Y. Kakoto, W. Watanaba, M. (KYOWA HAKKO KOGYO KK ,KYOWA HAKKO FOOD SPECIALTIES CO LTD, KYOWA HAKKO FOODS KK)	EP914777-A; EP914777-A1; AU9865990-A; CN1216686-A; JP11215956-A; CA2237699-A1; NZ330425-A; US6066352-A; KR99044734-A; (All 1999) SG77635-A1; 2001 AU757530-B; 2003 EP914777-B1; 2004 DE69825542-E; 2004 CN1515164-A; 2004 DE69825542-T2; 2005 CN1204822-C; 2005	Protein complex useful as emulsifier and flour improver.	A complex of a protein with a saponin or a bile acid is claimed. USE - An emulsifier (and emulsified compositions), a dough improver (and the dough and bread prepared from it) are all claimed. The emulsifier may be used in foods (e.g. mayonnaise, soup, oil-containing seasonings), pharmaceuticals (e.g. urea cream, acne cream), cosmetics (e.g. cream, milky lotions, foundation) and toiletries (e.g. shampoo, rinse)…Acidic and neutral emulsified compositions having excellent storage stability at room temperature and heating stability can be prepared. In food compositions, there is little deterioration in the taste of the food. The protein is wheat protein, soybean protein, plasma protein, albumin protein…

Inventor Assignee	Patent Number	Title	Details
	CN1307885-C; 2007 CA2237699-C; 2007 JP2007300929-A; 2007 KR565160-B 2006		
Tokaev, E. S Rogov, I.A Englin, I. P (MOSCOW MEAT DAIRY INST)	SU1353410-A 1987	Dietary protein emulsion for enteral probe nutrition - comprises malting, vegetable oil, meat-fish protein, vitamin(s), minerals and water.	Protein emulsion for enteral probe feeding, or for normal nutrition, contains a protein source, fat, carbohydrates, minerals, vitamins and water. The emulsion is more stable against , separation, provides more balanced and biologically valuable nutrients, if it is made from the following (wt. per cent): malting 11.25-22.5, fat, i.e. salad or olive oil, or a cross-esterified mixture of vegetable oil and bone fat 2.5-7.5, protein, i.e. egg, milk, meat, fish, or a milk protein-wheat gluten mixture, or soya protein isolate, or blood protein, or casein hydrolysate 2.5-7.5, vitamins 0.015-0.052, minerals 0.29-0.53, and balance water.....
Tokaev, E.S Rogov, I. a. Englin, I. P (MOSCOW MEAT DAIRY INST MEAT IND RES INST)	SU1353409-A 1987	Prodn. of emulsified product for enteral feeding - by mixing molasses, with or without pectin, casein hydrolysate, proteins, vitamin(s), etc. to produce stable liquid feed.	The aq. phase contains sources of carbohydrate, protein, with prodn. of a water/oil emulsion. Materials used include: low-saccharose-contg. molasses (with 10-30% reducing substances) or its mixt. with pectin. It is dissolved in water (to prod. aq. phase) with subsequent addn. of casein hydrolysate or blood protein, or mixt. of vegetable hydrolysates and animal proteins with unhydrolysed proteins
CHIBA FLOUR MILLING CO LTD JIBASEIHUN CO	AU8545915-A; 1986 JP61100176-A; 1986 JP88005065-B	Adhesive for bonding pieces of food - contg. protein of polysaccharide, and calcium material or sodium hydroxide coated	An adhesive compsn. for combining pieces of food contains (a) a protein, or decomposition prod., with at least 30 mols. COOH/10 power 5 g, and/or a polysaccharide, or decomposition prod., with at least 0.3 mols COOH/polymerisation unit, these being sol. or dispersible in

Inventor Assignee	Patent Number	Title	Details
	1986; KR8903742-B	with oil, fat, acid, surfactant, saccharide or gum.	water, and giving an aq. soln. or dispersion which is gelatinisable at less than 30% solids concn., and (b) calcined powder of egg shell, seal shell and bone, alkaline earth metal (hydr)oxides and/or NaOH, mixed or coated with oil, fat, aliphatic acids, surfactants, saccharides and natural gums. Pref. compsn. contains egg white, plasma protein and lactalbumin, with NaOH…Odd pieces of food can be utilised efficiently.
MEIJI MILK PROD CO LTD	JP60184366-A; 1985 US4626444-A; 1986 US4632840-A 1986	Water-in-oil-in-water emulsified dressing prepn. - by forming water-in-oil emulsion and phase converting to water-in-oil-in-water emulsion by stirring.	W/o/w-type cpd. emulsified dressings are prepd. by (a) preparing w/o-type emulsion either by adding water or aq. phase in oily phase and mixing or by adding oily phase in water or aq. phase and mixing them, and (b) phase-converting w/o-type emulsion to w/o/w-type cpd. emulsion by stirring…In aq. phase protein (e.g. casein, sodium caseinate, gelatin, wheat protein, soybean protein, serum protein, whey protein, white, yolk, etc.) starch (e.g. starch, processed starch, dextrin, starch derivative, etc.), gum (e.g. locust bean gum, xanthan gum, pullulan, dextran, tamarind gum, agar, carrageenan, etc. …
Mieth, G Kroll, J. Pohl, J. Brueckner, J. Gassmann, B. Schwirksli, K. Paul, W. (AKAD WISSENSCHAFTEN DDR)	DD150539-A 1981	Stable emulsion for human and animal nutrition - prepd. by emulsifying fat and water with preliminarily prepd. protein-lipid simplex emulsifier.	Prodn. of foodstuff emulsions comprises by (a) subjecting plant, microbial or animal protein solns. or suspensions to a tribochemical conversion under high mechanical shearing forces or high frequency vibrations, in the presence of surface active lipids, at pH above or below the isoelectric point of the main protein fraction and at low ion concns. of water-soluble electrolytes, esp. of neutral salts, e.g. NaCl. (b) The aq. medium is then emulsified with added fats. The proteins are esp. oil seed-, legume-, cereal-, milk-, fish-, yeast-, egg-, whey- and/or plasma proteins or their modifications, pref. in acid or heat-denatured form. ...Stable food emulsions are prepd. suitable for human and animal notrition. Specific uses include : simulated milk, mayonnaise, margarine, butter, cream, lard, whipping

Inventor Assignee	Patent Number	Title	Details
			cream, etc.
Stancu, V. Mosanu, C. INST CHIM ALIMENTARA BUCURESTI INTR PREPARATE CONSERVE DIN CARNE	RO105535-A 1992	Textured food prods. - prepd. from whole, stabilised blood or blood plasma by coagulation with calcium salts, sodium chloride, spices, nutmeg and garlic.	Textured food prods., based on blood, are prepd. from whole, stabilised blood, blood plasma or other sepd. constituents, by coagulation with 0.3-0.5% calcium salts, 1-2% sodium-chloride; 0.1-0.3% spices as pepper, nutmeg and garlic. The prod. is homogenised, transferred into corresponding stainless tubes, forming layers of 10 cm. max. thickness, and heat treated at 85-90 deg.C for 100-150 mins., having placed the tubes in conventional heating compartments.
Hart, J. R Thevenot, R. SOC PROD NESTLE SA HART J R NESTLE SA	EP651948-A; EP651948-A1; NO9403699-A; AU9474288-A; CA2133502-A; ZA9407577-A; JP7184600-A; NZ264599-A; AU681429-B	Continuous prodn. of solid food prods. based on blood - by thermal treatment of homogenised liq. mixt. of blood, lipid(s), water and protein source and/or hydrocolloid(s).	Continuous prodn. of a solid food prod. with a moisture content of 35-75% comprises thermal treatment of a homogenised mixt. of blood, lipids and water to which a protein source and/or hydrocolloids have been added before or after homogenisation. Pref. the mixt. comprises 20-50% lipids, 30-60% blood, 0.1-20% protein source and/or 0.1-2% hydrocolloids. Pref. thermal treatment is carried out in a tunnel oven at ca. 100C for 30 sec.-5 mins., or in a refiner-texturiser at 70-110C for ca. 30 sec.-5 min. The blood used is e.g. blood of pigs, cows, veal, etc., haemoglobin, plasma, etc… The protein source is e.g. blood plasma, soya protein, fish, offal (lungs, liver, kidneys, etc.) or casein. The hydrocolloid is ..e.g. konjak, guar, kappa-carrageenan, xanthan or mixts. of these. USE - The prods. are esp. useful as food for domestic animals. ..Homogenisation causes masking of the colour of the prod. since the blood acts as a stabiliser for the lipidic micelles which are sufficiently small to reflect a high proportion of incident light. The presence of the protein source and/or hydrocolloid gives a solid prod. with a stiff gel texture that can be cut into solid chunks and incorporated into terrines, gravy, etc. and stabilised before consumption e.g. by sterilisation, freezing or refrigeration.

Lee, J. H	US2004081725-A1 2004	Preparing fat and animal blood protein encapsulated product involves mixing fat ingredient with animal blood protein ingredient, heating the mixture, and adding peroxide to change the intense dark color.	Preparation of a fat and animal blood protein encapsulated product involves mixing a fat ingredient with an animal blood protein ingredient, and heating the mixture to above 55degreesC to form a fat-protein encapsulated gel product and adding a peroxide to change the intense dark color. USE - The method is used for preparing a fat and animal blood protein encapsulated product, animal plasma or serum proteins into two functional protein products of albumin fraction and immunoglobulin fraction, or protein fractions for fractionating liquid soy, rice, yeast, egg, milk, and whey proteins. The albumin fraction product is used for applications of binding and gelling purposes, non-toxic glue, nutrition, and fat-protein encapsulation. The immunoglobulin fraction product is used as a nutritional ingredient. (all claimed)

4.5 Modification of the functionality of bovine blood plasma

The previous sections have described the functional properties of blood components and discussed the potential applications of blood constituents as food ingredients, as found in the research and patent literature. This section focuses on techniques to improve functional performance and in particular, the foam stability of whipped bovine serum albumin in the presence of sugar as an egg white substitute and for a number of other functionalities.

A novel approach to plasma processing

Research work conducted at the Meat Industry Research Institute of New Zealand in the late 1970s (Mawson and Collinson - not published in the public domain) worked on the development of a cold-set binder for restructured meat products based on whole bovine blood plasma. The concept involved collecting the blood directly from hanging animals immediately post stunning using sodium citrate as the calcium binding anticoagulant. The blood was separated using a red blood cell centrifugal separator within 30 min of collection and the plasma concentrated to 20% protein using ultrafiltration. In addition to concentrating the plasma, ultrafiltration removed much of the calcium citrate and surplus sodium citrate so that only a low concentration of calcium ions needed to be added back to trigger the clotting reaction. The ultrafiltered plasma was soft frozen using an ice cream freezer purged with nitrogen and could be stored at -20°C for at least 12 months without losing clotting or seemingly at least, some of the anti-microbial functionality. In practice, this material was blended with Comitrol® flaked, tempered, frozen meat and a small amount of a source of slow release calcium ions and formed into steaks etc. The restructured meat could be packaged and distributed in a chilled or frozen form that retained the natural meat colour attributes and broke the 3 month storage barrier for frozen, restructured meats. This discovery was not transferred to industry. Additionally, the synthesis of a meat analogue made from the ultrafiltered plasma by slowly whisking it with the slow addition of dilute calcium lactate solution was explored. The fibrinogen formed a fibrous structure that could be mixed with a vegetable oil-based emulsion (using some of the residual serum as the emulsifier) and flavourings then heat set into a cooked meat analogue. A likely inhibitor of the uptake of the functional properties of blood plasma in non-meat products is the strong hay/grassy smell of the serum. Nevertheless, as discussed later, there are likely to be non-solvent extraction methods for removing these aromas.

Bovine serum prepared following the extraction of the fibrinogen proteins by precipitation will almost certainly be a mixture of bovine serum albumin and bovine serum globulin. In a comparative study of the functional properties of bovine blood proteins, serum albumin showed the best foaming properties, followed by whole plasma, globulin and globin. These proteins in 1% solution were able to emulsify 60%, 115%, 70% and 45% of their volume of oil, respectively (Vuono et al., 1979).

While most of the following discussion will centre on bovine serum albumin, another significant protein in blood plasma is bovine serum globulin. In general it has somewhat poorer functional properties compared to bovine serum albumin and ways of improving its functionality do not appear to have been widely canvassed. Nutritionally, bovine serum globulin has a higher essential amino acids index than bovine serum albumin (Prata and Sgarbieri, 2008). More specifically, it is a good source of lysine, leucine and valine; limiting amino acids are methionine and isoleucine. The protein has excellent foaming and emulsification properties. Mayonnaise-type emulsions are stable over 8 weeks storage at 4°C. Results indicate the potential of this globin protein as a relatively good emulsifying or foaming agent for incorporation into food formulations (Shahidi et al., 1984).

The nature of bovine serum albumin

Bovine serum albumin is a globular protein that has the tendency to aggregate in macromolecular assemblies. Its three dimensional structure is composed of three domains, each one formed by six helices, and its secondary structure is essentially α-helical. At a pH of 7, bovine serum albumin has about 10 effective negative charges per protein molecule. At room temperature, tertiary structure is well defined and stabilised. As temperature increases, some molecular regions become accessible to new intermolecular interactions, producing pH-dependent soluble aggregates through disulphide and non-covalent bonds (Militello et al., 2003). The sulphydryl-disulphide exchange underlies many protein functions in processed foods. It has been found that this interchange is particularly active in bovine serum albumin solutions compared to other globular proteins leading in particular to instabilities in the presence of metal ions (Owusu-Apenten et al., 2003). Additionally it has been reported that during the bovine serum albumin aggregation process, an important role is also played by liquid-liquid demixing due to the thermodynamic instability of the solution. Bovine serum albumin aggregation appears to be the result of three interconnected mechanisms: critically diverging concentration fluctuations associated with liquid-liquid demixing, conformational changes and protein cross-linking.

However, the hierarchy of these mechanisms is strictly dependent on the experimental conditions, evidenced by temperature scan measurements of static and dynamic light scattering and circular dichrosim (Militello et al., 2003).

In the experimental conditions of temperature and concentration, conformational changes at secondary and tertiary structure level are the driving step for a rapid initial intermolecular cross-linking process while, where secondary structural changes are prevented by blocking the free Cys-34 (for example by binding a fluorescent marker dye), intermolecular bonds are inhibited and the aggregation is slower and proceeds only through conformational changes at tertiary structure level (Militello et al., 2003).

The isoelectric point (pI) of bovine serum albumin is reported to be around pH 4.7-5.2 (Kongraksawech et al., 2007). Bovine serum albumin is particularly susceptible to denaturation at reduced pH. However, free fatty acids have been found to be protective against thermal denaturation (Relkin, 1996).

Thermal aggregation kinetics in D_2O solutions of bovine serum albumin were studied to investigate which type of structural and conformational changes are involved in the aggregation processes of bovine serum albumin. The tertiary conformational changes were followed by monitoring the Amide II band, the secondary structural changes and the formation of beta-aggregates by the Amide I' band and, finally, the hydrodynamic radius of aggregates by dynamic light scattering. The results show, as a function of pD, that: tertiary conformational changes are more rapid as pD increases; the aggregation proceeds through to formation of ordered aggregates (oligomers) at pD far from the isoelectric point of the protein; disordered structures add as the pD decreases. Moreover, beta-aggregates seem to contribute only to oligomers formation, as showed by the good correlation between kinetics of scattering intensity and IR absorption intensity. These results indicate for bovine serum albumin a general mechanism of aggregation composed by partial unfolding of the tertiary structure and by the decrease of alpha-helix and random coil contents in favour of beta-sheet aggregates. This mechanism strictly depends on pD, and gives rise to almost two distinct types of macromolecular aggregates (Militello et al., 2004). The formation of beta-sheet aggregates in proteins destined for human consumption is to be avoided due to the propensity of these structures to trigger the formation of like structures in other proteins known to be hazardous for human and animal health i.e. prion an amyloid like structures (Stathopulos et al., 2004). In other words bovine serum albumin should not be modified under high pH conditions or the use of high intensity ultrasound, which can also produce beta-sheet structures from bovine serum albumin (Stathopulos et al., 2004).

The addition of NaCl to the bovine serum albumin solution at neutral pH increased the gel strength by decreasing the electrostatic repulsion between proteins and the simultaneous presence of both sodium and calcium ions further strengthened the bovine serum albumin gel. The effects of very low concentrations of $CuSO_4$, $FeSO_4$, $ZnSO_4$ and $MgSO_4$, on the structure of bovine serum albumin gels have been investigated by transmission and scanning electron microscopies (Donato et al., 2005; Haque and Aryana, 2002). The comparison with pure bovine serum albumin gel has shown that the addition of $CuSO_4$ markedly changed the microstructure of bovine serum albumin gels: significantly larger water entrapping void spaces were seen and the gel matrix was comprised of larger aggregates. The same authors observed that in the presence of Zn^{2+}, aggregates were more compact and appeared less clustered and fused together than in the presence of Cu^{2+}.

The biological nature of bovine serum albumin

Serum albumin is an abundant protein in blood plasma, which is well-known for its ability to transport hydrophobic biomolecules. Recent hypotheses propose that serum albumin plays a role in the regulation of lipid metabolism in addition to its lipid transport properties. Bovine serum albumin has the capacity to extract diacylglycerols from phospholipid bilayers, but the process can be inhibited by divalent cations. Quantitative measurements using radioactive diacylglycerols and morphological evidence derived from giant unilamellar vesicles examined by confocal microscopy provide concurring results. Bovine serum albumin extracts diacylglycerols from vesicles composed of phosphatidylinositol and diacylglycerols. Long, saturated diacylglycerol species are attached to the bovine serum albumin more readily than the shorter-chain or unsaturated ones. Divalent cations hinder diacylglycerols uptake by bovine serum albumin. The concentration of Ca^{2+} causing half-maximal inhibition is approximately 10 µM; 90% inhibition is caused by 100 µM Ca^{2+}. To demonstrate how diacylglycerols uptake by bovine serum albumin and its inhibition by Ca^{2+} could play a regulating role in lipid metabolism, a PI-specific phospholipase C was assayed in the presence of bovine serum albumin and/or Ca^{2+}. Bovine serum albumin activated the enzyme by removing the end-product diacylglycerol, but the enzyme activation was prevented by Ca^{2+} ions which inhibited diacylglycerol uptake (Ahyayauch et al., 2009).

"Molten states" in partial thermal denaturation and their impact on functionality

In an early review of the significance of the partially molten state of globular proteins with respect to the functionality, it was demonstrated that by manipulating the oxidised and reduced states of disulphide bridges, of which there are 17 in bovine serum albumin, that the protein can enter what was then known as the molten globular state. The potential thermal development of the molten globular state was speculated but not discussed in particular for bovine serum albumin (Hirose, 1993).

A comprehensive review (McClements, 2002) explores the molten state or meta-stable states of proteins beyond the native state in depth. The issues are pointed out and extend beyond the simple manipulation of disulphide bonds or partial thermal denaturation and relate to all aspects of processing and ingredient formulation. Sugars, and sucrose for example, most likely acts as a stabilising co-solvent for bovine serum albumin at high concentrations while at low concentrations it could have the opposite effect. Under some conditions, it can act as a destabilising agent for egg albumen, while under others, it acts as a protectant. While preventing bovine serum albumin from denaturing at the gas solvent interface, it can promote egg albumen destabilisation. Under other circumstances, it can prevent destabilisation of the egg albumen at the solvent gas interface. However, it should be pointed out that whether co-solvent is stabilising or not stabilising is strongly contingent on many factors; for example, it may be stabilising at low temperature and actively destabilising at elevated temperatures. It is therefore a limitation of most foam stability evaluations that they evaluate the foam expansion and stability at ambient conditions to determine whether or not bovine serum albumin would work more effectively than egg albumen in a baking situation. The foam should really be cooked and then evaluated or preheated to a partially denatured condition then whipped then cooked. The review concludes with the observation that many advances in understanding thermo-physical and biochemical characteristics of biomolecular systems have occurred and that food science would do well to make use of this knowledge in assessing how particular proteins are functional or not functional in the presence of weakly interactive co-solvents and how they can be made functional, given a set of application circumstances.

The effect of heat treatment on the protein, below the denaturation temperature, studied by dynamic light scattering and infrared spectroscopy measurements, led to the formation of small aggregates (which were the constituent units of the larger aggregates present in the gelled state). After cooling the sample and addition of Cu^{2+} or Zn^{2+}, a cold-set gel is produced. Sustained heating below the denaturation

temperature produced small aggregates of about 20 nm in size, originating from the conversion of native α-helices into β-aggregates. The heated bovine serum albumin solution had a weak "solid-like" character. The cold-set gel following the cation addition exhibited a highly elastic character a few minutes after the metal ion addition. The elastic and viscous moduli strongly depended on metal ion concentration and two regimes can be distinguished below and above 10 mM. This behaviour supports the hypothesis that two effects play an important role in the network formation: the shielding action exerted by ions against the charges present on the protein surface and the "bridging" effect due to the ability of the ion in coordinating several oligomeric structures. Of the two metal ions, Zn^{2+} produced the firmer gels (Navarra et al., 2009) and would be more acceptable in a food system.

The problem with BSA as a foaming agent for foods and potential solutions

It is perhaps curious that bovine serum albumin is such a powerful emulsifying protein compared to other globular proteins but does not perform as well in terms of foam expansion or stability, as both phenomena rely on the denaturation of the protein at the interface between a hydrophilic and hydrophobic substance (Mitchell, 1986). But as noted above (McClements, 2002), denaturation is a complex phenomenon and it does not necessarily follow that this is true. The most likely explanation is the propensity of bovine serum albumin to form ligands with lipids which are known inhibitors of foam formation (Nino et al., 1998). The distribution of proteins and lipids in food emulsions and foams is determined by competitive and cooperative adsorption between the two types of emulsifiers at the fluid-fluid/gas interfaces, and by the nature of protein-lipid interactions, both at the interface and in the bulk phase. The existence of protein-lipid interactions can have a pronounced impact on the surface rheological properties of these systems. The attraction of protein and lipid to the interface in a foam or emulsion is a complex competitive process (Patino et al., 2001). In an investigation of the surface activity of bovine serum albumin; surface data at low protein concentration indicated a low surface activity, which rose to a plateau as the molecular monolayer became saturated at higher protein concentration. Protein concentration and surface tension at the plateau depended on the aqueous phase composition. The effect of the aqueous phase composition on bovine serum albumin-lipid interactions was also investigated by spreading an insoluble lipid (monostearin or monoolein) on a film of bovine serum albumin previously adsorbed on the interface. The existence of protein-lipid interactions depended on the protein/lipid ratio. At low ratios, surface activity of mixed bovine serum albumin-lipid films was determined by the lipid since the surface

pressure of the mixed film was the same as the lipid equilibrium spreading pressure, and the monolayer was not saturated by bovine serum albumin. However, the surface activity of mixed bovine serum albumin-lipid films at higher ratios was determined by bovine serum albumin as the monolayer became saturated by the protein (Rodriguez-Nino and Rodriguez-Patino, 1998).

Careful studies of the denaturation characteristics of bovine serum albumin in the absence and presence of sucrose by micro-DSC and rheology were made of bovine serum albumin solutions containing 10 mmol sodium chloride, with and without added sucrose. Sucrose levels ranged from 0 to 40% by weight. While sucrose increased the thermal stability with respect to denaturation temperature it also decreased the denaturation enthalpy, which was attributed to a departure from native structural format of the protein induced by the presence of sucrose. It was also noted that the presence of sucrose generated irreversible denaturation of the thermally denatured protein compared to bovine serum albumin solutions without added sucrose. The combination of these effects was responsible for a decline in the sheer modulus after 15 minutes holding time at 90°C. However, after holding for two hours the strength of the protein gel increased due to a greater fraction of the irreversibly denatured protein that was formed. The implications of this latter observation may be relevant to the concentration of bovine serum albumin needed in order to obtain maximum functional performance in foams and gel structures in the presence of sucrose (Baier and McClements, 2001).

In an investigation of the kinetics of bovine serum albumin foam instability, the instability of the foam generally followed either monophasic or biphasic first-order kinetics, which implies that two microscopic processes, namely, gravitational drainage and interbubble gas diffusion, are fundamentally responsible for protein foam instability. The relative magnitude of the rate constants of these two processes is affected by environmental factors such as temperature, pH, and the presence of reducing agents (Yu and Damodaran, 1991).

A number of strategies to improve the stability of bovine serum albumin foams have been investigated:

- The foam stability properties of a mixed solution of Tween 20 and bovine serum albumin were evaluated as a function of arabinoxylan concentration (using a soluble wheat arabinoxylan fraction). A marked increase in foam stability was observed with low concentration of arabinoxylan. A maximum improvement in the foam stability was obtained with 0.2-0.3 mg/ml of arabinoxylan. Enhancement of

foam stability was identified as a combination of bulk viscosity changes and surface effects. The relative contribution of arabinoxylan to bulk viscosity and adsorbed layer structure was studied by examination of the properties of thin liquid films at the macroscopic air-water interface. Arabinoxylan reduced the rate of thin film drainage, increased the equilibrium thickness of the films, slowed the lateral diffusion of a fluorescent probe molecule located in the adsorbed layer, and increased the surface elasticity. These data are congruent with arabinoxylan-mediated crosslinking of adsorbed protein (Sarker et al., 1998).

- Combinations of basic (high pI) and acidic (low pI) proteins exhibited tolerance to lipids that are normally potent inhibitors of foaming. Bovine serum albumin with the basic protein clupeine (a protein from herring roes) (pI 12) foamed effectively even with 25% (v/v) maize oil present, although the tolerance level for L-alpha-lecithin was much lower (Poole et al., 1986). Addition of 0.5% clupeine to powdered beef plasma increased overrun (foam volume) and foam stability by 43.7 and 127.3%, respectively (Seung-Taek and Jeong-Min, 1995).

- The attachment to the hydrophobic amino acid residue valine to the lysine residues of bovine serum albumin significantly improved both the whipping and gelling properties of bovine serum albumin compared to the untreated protein. Interestingly the apparent hydrophobicity of the protein only increased after the thermal denaturation of the protein (Murphy and Howell, 1991b). Further investigation of enhancement of lysine groups by attaching lysine, via the carboxyl groups of bovine serum albumin on its physicochemical and functional properties were conducted. The modified samples had high isoelectric points and were highly positive when separated electrophoretically. Partially and extensively modified samples behaved differently in their pH titration curves. The gelation and whipping properties of these lysyl derivatives, in partially modified samples had high values compared with the control and in extensively modified samples had lower values. Two explanations are suggested; either there is increased electrostatic attraction between molecules whose isoelectric points are closer to neutrality, or there is a midpoint where a partial degree of conformational disruption results in superior functional properties (Murphy and Howell, 1991a).

- There may be the potential to use the strategic addition of phenolic compounds such as tannic acid, caffeic acid and ferulic acid to bovine serum albumin foams to improve foam stability. The addition of these compounds to porcine plasma protein-based films improved the structural strength (Nuthong et al., 2009). The interactions between a monomeric phenolic compound (caffeoylquinic acid) and

bovine serum albumin appears to be non-covalent and has no impact on the stability of bovine serum albumin in solution (except at low pH) and is somewhat protective against thermal denaturation (Prigent et al., 2003). For a detailed review of the chemical interaction between phenolic compounds and proteins see Rawel and Rohn (2010). It appears the non-covalent interaction observed in these studies is as much a function of the hydroxycinnamic phenolic acids as it is of the protein, i.e. the choice of phenolic compound is important in determining the outcome.

In a study exploring the potential for targeted delivery of hydrophobic drugs on microbubbles that form ligands with bovine serum albumin, it was found that after ultrasound, more than 90% of the entrapped bovine serum albumin was released from the drug and the microbubbles (Lu et al., 2010). Release of the bound lipid associated with bovine serum albumin may also be obtainable by pH adjustment in the presence of microbubbles rather than by direct application of ultrasound. The bovine serum albumin molecule consists of a chain of 582 amino-acid residues, forming a single polypeptide, which contains three homologous a-helix domains (I-III), which are divided into nine loops by 17 disulphide bonds. Its primary structure is unusual in possessing a single sulfhydryl group (Cys-34). Every domain can be divided into 10 helical segments. The molecule is not uniformly charged within the primary structure. Bovine serum albumin is the transporter of cholesterol, fatty acids and anions via the blood. Above the iso-electric pH 5.0, bovine serum albumin is negatively charged and the interaction with phospholipids is by hydrophobic interaction, not by electrostatic interaction (Torrens et al., 2009). Below the iso-electric point, there is potential to release the bound phospholipid onto the surfaces of micro-bubbles (Lu et al., 2010) through the loss of association with the now anionically charged bovine serum albumin molecule which favours electrostatic interaction.

Bovine serum albumin proved to be a particularly suitable protein for concentration and separation by foam stripping. In part because of its high surface active characteristics and enrichment factor and in part because of the requirement of very low gas flows in continuous separation systems. This characteristic augurs well for the separation of bovine serum albumin from waste water streams resulting from meat processing (Monwar Hossain and Fenton, 1998).

Modification of BSA for other functional purposes

While the lipid binding propensity of bovine serum albumin may be a problem for foam stability it can also be exploited for the extraction and stabilisation of hydrophobic nutraceuticals into aqueous environments. Functional foods represent an emerging market of growing economic importance. The formulation of carotene-fortified food emulsions involves the difficulty of transferring the carotenoids into the lipid phase. In a novel approach the lipid binding capacity of bovine serum albumin was exploited. The carotenoid was first bound to bovine serum albumin and then the carotenoid-protein complex was used to prepare an emulsion. Absorbance spectroscopy indicated the formation of the complex in the aqueous phase and provided the first evidence of the carotenoid load, which subsequently formed an emulsion as supported by the colour and confocal microscopy of the cream phase (Wackerbarth et al., 2009)

In a similar vein as previously noted (McClements, 2002), the binding of flavour ligands modify the thermodynamic characteristics of proteins during denaturation. It is determined that bovine serum albumin binds the flavour compound vanillin more strongly than either ovalbumin or beta-lactoglobulin. Bovine serum albumin was annealed through a series of temperature steps and it was found that the affinity of bovine serum albumin for vanillin is decreased in the order: native protein greater than denatured monomer greater than denatured clusters. The interaction of vanillin with the proteins is mainly electrostatic in nature (Mikheeva et al., 1998).

It has been shown that heating solutions of globular proteins such as bovine serum albumin in the presence of low molecular weight reducing sugars, such as lactose, ribose, or xylose causes the formation of gels that show higher breaking strengths than comparable samples heated in the absence of reducing sugars. Furthermore, the amount of protein required to form a gel can be reduced by up to 3-fold and the time required to form the gel is also reduced. Similarly the emulsifying properties of bovine serum albumin can be improved through the combination with low molecular weight reducing sugars (Oliver et al., 2006). There does not appear to have been any observations of the impact of low molecular weight reducing sugars on the foaming properties of bovine serum albumin. These same reactions can be used to synthesise lipid antioxidants although the identity of the responsible compounds is ill-defined (Zamora and Hidalgo, 2009). But if at least some of these compounds can be formed from bovine serum albumin without compromising its ability to form lipid ligands, there is the potential to create a very effective lipid antioxidant from bovine serum albumin. The antioxidative activity of nonenzymatically browned bovine serum

albumin produced by reaction with ribose, hydroperoxides of methyl linoleate oxidation, and secondary products of methyl linoleate oxidation, at different pHs (4, 7, and 10) and temperatures (25, 37, 50, 80, and 120°C), was studied to compare the antioxidative effects of carbohydrate- and oxidized lipids-modified proteins. The modified proteins ribose-bovine serum albumin, hydroperoxide-bovine serum albumin, and secondary-oxidation-bovine serum albumin were tested for antioxidative activity (at 100 ppm) in soybean oil using the thiobarbituric acid-reactive substances assay. All of them decreased significantly ($p < 0.05$) the thiobarbituric acid-reactive substances formation in the oil and exhibited different effectiveness as a function of the temperature and the pH of the medium. In addition, there was a good correlation between the antioxidative activity of the protein and the amino acid losses produced during the nonenzymatic browning (Alaiz et al., 1999).

Bovine serum albumin was phosphorylated by two methods: by dry-heating in the presence of pyrophosphate; and by conjugation with maltopentaose, through the Maillard reaction, and subsequent dry-heating in the presence of pyrophosphate. The phosphorus content of bovine serum albumin was increased to approximately 0.45% by dry-heating at pH 4.0, 85°C for 5 d in the presence of pyrophosphate, and approximately 0.91% by glycation and subsequent phosphorylation. The circular dichroism spectra showed that the change of secondary structure in the bovine serum albumin molecule by phosphorylation was mild. However, tryptophan fluorescence intensity of bovine serum albumin decreased by phosphorylation. The differential scanning calorimetry thermograms of bovine serum albumin showed a disappearance of the 1st denaturation peak and a temperature lowering of the 2nd peak by phosphorylation. These results indicated that molten (partially unfolded) conformations of bovine serum albumin were formed by both phosphorylation methods. The functional properties of bovine serum albumin, such as heat stability and calcium phosphate solubilising ability were improved by phosphorylation alone and further by phosphorylation after glycation. Transparent bovine serum albumin gels with relatively high water-holding capacity were obtained by phosphorylation alone and following glycation and phosphorylation. The immunogenicity of bovine serum albumin was reduced significantly by glycation and phosphorylation, respectively (Enomoto et al., 2008).

The addition of α-lactalbumin, which of itself does not gel at temperatures up to 80°C, to bovine serum albumin solutions enabled the formation of firmer and more transparent gels than with bovine serum albumin alone. The enhancing effect of α-lactalbumin on the gel hardness of bovine serum was ascribed to the formation of a

finer more uniform gel matrix resulting from an interaction to form soluble aggregates between the proteins through thiol-disulphide interchange during general formation (Matsudomi et al., 1993). A similar synergistic effect is noted when bovine serum albumin is added to porcine serum albumin (Miyaguchi et al., 2001).

Other techniques for the modification of bovine serum albumin include partial enzymic hydrolysis, which most commonly has a negative impact on functional properties and gamma irradiation which also has a negative impact above 5 Gy and no impact below 5 Gy. For this reason these techniques are not considered in this review of blood plasma modification.

4.6 Factors that influence the of use of blood ingredients for functionality in food products

Based on the average Australian beef dressed carcase weight for 2010 of 272.1 kg and the yields of edible blood measured by Graham & McPhail (1974), approximately 8.7 kg could be collected per head in 60 seconds. Approximately 7,690,000 cattle were slaughtered in Australia in 2010 (MLA, 2010), therefore if blood for edible purposes was collected from all of these, there is the potential for 66,900 tonnes of edible blood to be available.

Marketing blood protein products to the food industry at large, will be an uphill battle because of the potential Yuk (and fear of BSE?) factors associated with the declaration of bovine blood proteins on ingredient labels in affluent Western markets. Other factors also impacting on use in the food industry are outlined below.

Hygiene

To enable blood from cattle and any of its products to be acceptable for human consumption, the blood must be collected in a hygienic manner. An anticoagulant must be added at the time of collection to enable the liquid blood to be processed into various products.

Blood for edible purposes may be collected by simply catching the blood flowing from the stick wound in an open bucket with an anticoagulant. However the microbiological quality of blood collected in this manner is generally poor. For blood to be collected hygienically at an abattoir, a much more sophisticated and costly system is required.

A system for a medium or large abattoir can consist of a carousal equipped with hollow-handled knives which are inserted close to the heart of the cattle or pigs. The blood with anticoagulant added flows from the knife through a system of tubes to collection tanks. A series of tanks is normally installed to allow batches to be held until the animals have been passed for edible consumption (Graham & McPhail, 1974). The blood is then separated into plasma and red cells which can be dried or frozen or further fractionated. This is quite a sophisticated and expensive system in which very few abattoirs in Australia have invested. Therefore there is currently a limited quantity of high-quality edible blood available in Australia.

To provide marketing flexibility, an increasing number of Australian plants are slaughtering cattle according to a certified procedure that meets the requirements of

the Halal market. This involves a transverse cut of the animal's neck to sever the carotid arteries. This is often followed by a thoracic stick to enable complete bleeding. It is somewhat more difficult to collect blood for edible purposes under this system although it has been demonstrated that collection of blood of acceptable microbiological quality is achievable. Currently 14 of the 17 major abattoirs in Queensland are certified for Halal slaughtering of cattle.

Cultural taboos

There are many people in the world who's cultural or religious beliefs prohibit them from consuming blood or products containing blood or blood fractions. These include Jews, Muslims and some Christian sects such as Jehovah's Witness. The Jewish Torah prohibits consumption of blood. Leviticus, Chapter 7 v 26 states: '*And ye shall eat no manner of blood, whether it be of fowl or of beast, in any of your dwellings.*' There are other verses in Chapter 17 that explain that blood is not eaten because the life of the animal is contained in the blood. This applies only to the blood of birds and mammals and not to fish blood.

The Islamic faith also prohibits the consumption of blood based on several verses of the Quran, including verse 5.3, which states: '*Forbidden to you (for food) are: dead meat, blood, the flesh of swine ...'*. Currently adherents to Islam comprise approximately 22% of the world population and numbers are increasing to the extent that it is expected to be the dominant religion in the world by the latter of 21st century. Judaism comprises less than 1% of the world population. (religioustolerance.org). Therefore close to 23% of the population of the world will not consume blood or blood product under current religious dogma.

Social implications and trends

A report (Protein Ingredients: A Global Strategic Business Report, www.strategyr.com/Protein_Ingredients_Market_Report.asp) by Global Industry Analysts Inc. (September 16, 2010) projects the global protein ingredients market to exceed US $24.5 billion by 2015. This steady growth is propelled by 3 major factors – an increasingly health-conscious population, growing scientific evidence supporting the advantages of proteins, and rising income levels, particularly in developing countries.

Europe represents the largest market for protein ingredients; the US, which accounts for more than one-fifth of the global protein ingredients market is projected to expand over the period 2010-2015 (annual average growth rate 8-9%).

Due to the changing perceptions of the growing health-conscious population, the consumption of plant proteins has increasingly gained momentum in the protein ingredients market. Besides this nutrition factor, the vegetarian movement is also contributing significantly to the growth of plant proteins. In particular, soy proteins are finding applications in several food products owing to their economic cost against animal proteins. The substitution of less physically functional, but less expensive plant protein ingredients for animal proteins in many areas of food manufacturing is boosting the soy protein market.

The plant proteins segment is forecast to take market share from the animal protein ingredients segment over 2010-2015. However, in contrast to the developed countries, demand for animal proteins is reportedly picking up in developing countries due to increasing population and rising income levels.

Global population growth and food security

It is predicted that global population growth will reach 9.1 billion by 2050. Concurrently food production, supplies of arable and water are predicted to decrease in the face of climate change induced resource constraints and urban growth. The approach of these challenges will increase the focus on food security across all food industries (Cribb, 2010).

The red meat industry could increasingly become a focus of climate change/ sustainability activists and will be required to demonstrate that optimum food yield is achieved from animals. When a broader range of protein sources are utilised for human food, the excellent physical functionality of blood proteins in structured and formed composite food products will be increasingly important.

4.7 Potential applications of blood plasma proteins in the food industry

Putting the issues influencing and/ or limiting the use of blood proteins in the food industry aside, there is clear potential for a number of non-meat food product applications if the proteins are modified by stripping them of potential off-flavours.

The easiest and still under-exploited market for unrefined bovine blood products is within the processed meat industry, particularly in Australasia. There is no reason, if the blood products are priced correctly, why they shouldn't completely displace the use of non-meat proteins and many other "binding" agents, heat or cold set, in this industry and simultaneously improve product texture, while providing scope for a cleaner label on these products. Red blood cells can be used to disguise fat pre-emulsions (or fat substitutes) in cooked emulsion products (Zayas and Zyrina, 1975).

For non-meat applications, it is critical that the blood plasma is separated from the whole blood before there is significant haemolysis, the plasma should be a light straw colour without a red tinge.

The next major market for defibrinated blood plasma (serum) is likely to be the bread baking industry as a dough improver in whole meal bread and, after complexing with arabinoxylan (a water soluble hemicellulose extracted from wheat bran), in white breads and gluten free breads. This application may be able to tolerate the flavours associated with blood plasma products.

For most bakery and confectionary applications, deflavoured blood serum could substitute directly for egg products and in some instances where emulsification is the dominant role, it should perform better than egg. Where gel strength/shortness is critical, some protein modification or higher usage level may be required. Where foaming is the principal attribute required, separated blood albumin may have the desired functionality after flavour stripping or further modification may be necessary, for example, by complexing with soluble hemicelluloses. For some confectionary applications, the transparency of bovine serum albumin modified by dry phosphorylation will provide an edge over egg albumen. Note that this is not a substitute for gelatine in confectionary as the bovine serum albumin gelation is irreversible. Modification of bovine serum albumin that enables cold-setting would be particularly suited to frozen confections such as gelati.

Deflavoured blood serum could directly substitute for egg and egg substitutes (if the price is right) in mayonnaise formulations.

There is increasing pressure on the food industry to remove chemical ingredients from their products, particularly those that are likely to persist in the body such as the antioxidants dehydroxy anisol (DHA) and dihydroxyl toluene (DHT), which opens opportunities for “natural antioxidants” such as those produced in the reaction between reducing sugars and bovine serum albumin.

Solvent extraction is being discouraged in the food industry and flavour-stripped plasma can be used as an extraction agent in aqueous systems.

Aroma stripped blood serum can also be a suitable feedstock for the flavour ingredient industry and may largely substitute for meat extract.

5 Conclusions

The results of the literature review showed that blood proteins have good functional properties including solubility, emulsifying, foaming and gelling properties. Since there are no standard methods for the determination of emulsifying and foaming properties, numerical comparison of reports from different studies as well as comparison with the properties of other proteins is difficult. However, the existing information in the scientific and patent literature suggests that blood proteins have comparable (and perhaps superior) functional properties when compared to 'traditional' protein ingredients such as egg white, whey protein concentrate, soy protein isolate and muscle proteins, and can be used as alternative ingredients in many processed food products. Applications including the use of plasma and its constituents as:

- emulsifiers in emulsion foods such as mayonnaise and spreads,
- gelling, water and fat binding and gluing (binding) agents in processed meat products,
- foaming agents,
- components of edible coatings and casings of sausages and other meat products, and
- microencapsulation delivery systems have been patented in different countries.

As with other proteins, the functional properties of blood proteins are highly dependent on the intrinsic properties of the proteins as well as extrinsic factors such as pH, ionic strength, method of isolation and drying technique employed. The available scattered data suggests that the functional properties of blood proteins are dependent on the origin of the blood. For instance, some differences between the gelling properties of bovine and porcine globin have been reported. Nevertheless, there is a lack of comparative studies on the effect of blood origin (bovine, porcine etc) and other factors such as breed, feed and climatic conditions on the quality and functionality of blood proteins. Limited information (in well defined systems) is available on the functionality of blood proteins compared to other proteins in both model systems and real food systems. Overall, plasma and its constituent proteins can find applications in a wide range of food products since their functional properties are maintained at a relatively wide pH and concentration range, whereas globin may be used as an emulsifying, texturising and foaming agent in acid food products (pH ≤6.0). However, it has to be noted that the functional properties of globin, and their dependence on pH and other parameters, are highly dependent on the method of

isolation employed to separate the protein from the haem fraction. These methods often use harsh chemicals such as hydrogen peroxide and acids which may cause different level of denaturation with varying consequence on functional properties. Less harsh methods of isolation may improve the functional properties of the protein at a wider pH and salt concentration range. Combinations with other blood components such as plasma have also been successfully used to improve the gelling properties of globin.

Before embarking on the development of an ingredients industry, the meat industry needs to evaluate the economics of recovering the available blood proteins to a food-grade hygiene standard. If the cost as an ingredient feed material in processed products is not lower than the price of currently used food ingredients taking into account usage level (at effective concentrations) then a higher a value market such as the pharmaceutical industry should be targeted.

- The processed meat and dietary supplements industries remain the major users of unmodified plasma components.
- For other food applications, de-flavouring is required, but plasma is potentially a valuable emulsifying and gelling agent and aqueous system extraction agent.
- To make the most of confectionary and all baking opportunities, pre-complexing with soluble hemicelluloses will overcome functional shortcomings with leavened and baked volume and foam stability.
- The creation of de-flavoured and more functional blood-derived ingredients requires product and process development based on a sound scientific understanding of the proteins and the physical and reaction processes required. Additionally, a similar understanding is required of the functional characteristics of the target food products where the blood derived ingredients will be utilised.

6 Accreditation of the reviewers

A number of authors of this review (Christine Margetts, Peter Roupas, Anita Sikes) are accredited reviewers for the Joanna Briggs Institute (JBI) and have undertaken "*Comprehensive Systematic Review*" training on "*Evidence Based Health Care and the Systematic Review of Evidence*", including a module on "*Systematic Review of Experimental and Non-Experimental Study*". The JBI is an initiative of the Royal Adelaide Hospital and the University of Adelaide and is an international collaboration involving medical and allied health researchers and academics across 40 countries in every continent.

7 About the authors

Dr. Netsanet Shiferaw Terefe is a research scientist at CSIRO food and nutritional sciences. Netsanet has a B.Sc. in chemical engineering, a Masters degree in food engineering from the Catholic University of Leuven (KUL), Belgium and a Ph.D. from the same university, which she completed in 2004. Over the last 10 years, Netsanet has been involved in fundamental and applied research on various aspects of food process engineering. Since joining CSIRO as a postdoctoral fellow, she has been involved in different research projects dealing with improving food product quality and processing efficiency through the application of innovative technologies such as high pressure, ultrasonics and pulsed electric field processing alone or in combination with traditional technologies. Her research activities over the last four years include:

- Application of ultrasonication for enhancing the kinetics of enzymatic hydrolysis of starch and other food biopolymers
- Combined high pressure-temperature inactivation/activation kinetics of quality degrading enzymes in different matrices
- Ultrasonic and combined ultrasonic and thermal inactivation kinetics of quality degrading enzymes
- Ultrasonic pre-treatment to improve the drying kinetics and functional properties of dried fruits
- Design and development of optimal processes to reduce the degradation kinetics of plant bioactives such as anthocynanins in food matrices during processing and storage
- Application of ultrasound for modifying the structure and functionality of plant fibres and other food bioplymers

Currently, she is working on the characterisation of temperature responsive polymers for separation of biomolecules from food streams as part of the separation team and on metabolomic understanding of ultrasonic assisted degradation of ligno-cellulose

biomass by white rot fungi. Netsanet is the author of several peer-reviewed research articles, book chapters and conference papers.

Mr Kirthi De Silva is a Chemical Engineer with considerable experience in leading and managing research and development projects for domestic and international food manufacturing clients. His main area of expertise is developing and scaling up separation technologies. Kirthi has been involved in many projects developing separation technologies including:

- commercialisation of the world's first continuous chromatography (CSEP) application in the food industry to manufacture whey protein isolate
- commercialisation of two further applications of CSEP for the production of high-value bioactive functional ingredients
- development of a cost-effective aqueous-based technology to separate chondroitin sulphate from marine sources (provisional patent)
- development of an aqueous-based platform technology to separate glycosaminoglycans from meat and marine waste streams (provisional patent)
- development of separations technology to produce functional ingredients from seed meal for a US-based multinational company
- development of a cost-effective technology to fractionate bovine plasma.

Dr Peter Roupas obtained his PhD from the Department of Medicine at Monash University, Melbourne, Australia in 1988 and completed his postdoctoral research at the University of Michigan Medical School, USA. During his 3 years at the University of Michigan, he was awarded fellowships from the American Diabetes Association (Michigan) and the Juvenile Diabetes Foundation International (New York). On his return to Australia, to the Department of Clinical Biochemistry at the Royal Children's Hospital, Melbourne, he was awarded the 1991 Eli Lilly Diabetes Fellowship and a 4-year fellowship from the National Health and Medical Research Council (NHMRC) of Australia. For the past 15 years, Dr Roupas has been a Research Team Leader at CSIRO and is currently a Project Leader of projects for the CSIRO Food Futures Flagship and the Preventative Health Flagship in functional foods relating to the scientific substantiation of health messages for dietary guidelines and health claims for food standards / regulatory applications. He is currently the Team Leader of the Knowledge Management team within the Pre-Clinical and Clinical Health Substantiation group within CSIRO Food and Nutritional Sciences. Dr Roupas has been an editorial reviewer for 8 scientific journals, an author of 44 papers in peer-reviewed scientific journals, 30 conference papers and 7 book chapters. Dr Roupas is also a Scientific Editor for Elsevier Science UK (*International Dairy Journal*), a

member of the Editorial Board of the *Journal of Functional Foods*, and a member of the *Society of Editors*.

Ms Christine Margetts has qualifications in librarianship, information management, writing and editing. She has over 25 years experience in providing information services to scientists and researchers working in agriculture, engineering and food sciences. As part of the Knowledge Management team within CSIRO-Food and Nutritional Sciences, she provides intensive information services in food and ingredient innovations to scientific groups. This includes developing in-depth literature searches and reviews, assistance with scoping studies and project reports and alerting researchers and business staff on scientific, marketing and intellectual property developments in the food industry.

Mr Neil McPhail has been involved in the area of Meat Industry Services at CSIRO Food and Nutritional Sciences for 10 years, providing technical advice to the meat industry in the form of Meat Technology Updates and responding to technical enquiries. Neil has contributed significantly to research projects of an engineering and technical nature and consulting assignments with the meat processing industry, including the use of abattoir waste heat for absorption refrigeration and cooling of hot offals. His main areas of expertise are in the fields of carcase and carton chilling and freezing, slaughtering and boning processes and solid waste handling and disposal. During the provision of these services and completion of projects, Neil has developed many contacts throughout the meat industry and has an extensive knowledge of most meat processing plant operations.

Ms Anita Sikes is a science graduate and project leader in the Food Chemistry and Biochemistry group at Coopers Plains, with 20 years experience in meat science relating to red meats. She has been involved in several red meat research areas funded by Meat and Livestock Australia. She has particular expertise in the role of collagen and connective tissues in muscle texture and processing, as well as expertise in strategic research at the micro-structural and biochemical level.

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