final report

Project Code: P.PSH.0415

Prepared by: Industry sponsor and MLA

Date published: September 2013

PUBLISHED BY
Meat and Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Utilisation of blood and blood components– Adding value to blood by using processes to separate and stabilise key components with the added benefit of removing blood from current slaughterhouse waste streams

This is an MLA Donor Company funded project

Meat & Livestock Australia and the MLA Donor Company acknowledge the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Executive Summary

Project P.PSH.0415 worked in collaboration with a food manufacturer to identify and develop potential uses for bovine blood and blood derived products. The project was undertaken in 5 stages. In Stage 1, a Research Provider was engaged to review blood opportunities and conduct testing and experimentation on blood and blood derived products. The risks of handling blood were also reviewed and taken into consideration. The project identified the production of **concentrated liquid plasma** (cLP) that may be lower in cost than currently available **spray dried plasma** (SDP) as an opportunity for exploration. Spray dried bovine blood plasma is used in the food manufacturing industry as a binder for a variety of products.

In Stage 2, technologies to microbiologically stabilise blood for storage and transport of blood and plasma with minimal refrigeration were investigated. In addition, technologies to separate plasma from red blood cells were examined and the functional properties of **single strength liquid plasma** (ssLP) were compared with SDP. In Stage 3, technological options to concentrate and microbiologically stabilise plasma were examined. In Stage 4, the laboratory processes were scaled up to pilot scale and the stability and functionality of the cLP examined. In Stage 5, larger quantities of blood were collected from an abattoir, separated using the pilot scale process developed and the cLP produced was successfully utilised by the food manufacturer. Proximate analysis of the final cLP is shown below.

A number of business models were analysed to determine the final cost of the cLP and the commercial viability of the process. Model 1 (the entire collection and processing is carried out at the abattoir) was found to be the most economical (see table below). As multiple assumptions were made, it is recommended that independent advice and costs be obtained to determine and validate the economic viability.

Proximate analysis (estimates based on trial production runs)

	Percentage
Protein	20-25
Fat	<0.5
Ash	<6.0
Carbohydrate	1-3
Moisture	65-75

Production costs

	Cost ($AUD)
Estimated Concentrated Liquid Plasma Production Costs	$540 per tonne*
Estimated Production cost per head of cattle	$0.97*

**multiple assumptions were made including but not limited to: production costs based on entire process located at the abattoir, abattoir processing 1500 head/day, and 5 days/week. It is recommended that individual abattoirs obtain independent economic analysis for their particular situation*

Contents

Utilisation of blood and blood components 1
Executive Summary 2
Purpose and Description 4
Summary of Project Stages 4
1.1 Stage 1 – Literature Review and Recommendations 4
1.2 Stage 2 – Production, Stabilisation and Use of Liquid Plasma 6
1.3 Stage 3 – Lab scale Proof of Concept 6
1.4 Stage 4 – Pilot Scale Production and Processing 6
1.5 Stage 5 – Commercial Scale Production and Processing 7
1.5.1 Blood Collection 7
1.5.2 Blood Volumes 7
1.5.3 On-Site Blood Collection 8
1.5.4 Blood Processing 8
1.5.5 Plasma Stabilisation 9
1.5.6 Applications of concentrated liquid plasma 9
1.5.7 Business models 9
Conclusion 10

Purpose and Description

Blood contains plasma (approx. 55%), red blood cells (approx. 45%) and white blood cells and platelets (1%). The protein content of whole blood is 18-19% with a portion of this captured in the plasma fraction. At present the majority of blood is classified as a waste stream. A small amount of whole blood is separated into plasma and red blood cells. This is low temperature spray dried and used in various food and feed applications. The aim of the project was to add value to blood. Following stage 1, the project focused on the use of blood to produce concentrated and stabilised liquid plasma and was undertaken in an additional 4 stages (Stages 2 to 5).

Summary of Project Stages

1.1 Stage 1 – Literature Review and Recommendations

The aim of stage 1 was to identify major risks with handling blood, to understand the composition of blood and its physical characteristics/functionality, ways to add value to blood, before finally determining the best option for separating blood into plasma and red blood cells. Below is a summary of *Stage 1.*

Risk factors in Handling and Processing Blood

Bovine blood, harvested for either human consumption or for further processing to produce bioactive agents, present multiple risks to collection workers. The risks from zoonotic agents is greatest during the early collection and processing stages, but present no greater risk than those encountered in routine beef production. Normal hygiene and safety measures used routinely in beef production should limit the chances of exposure to such agents. Only animals deemed fit for human consumption would be used in the collection of bovine blood.

Further separation of blood using centrifugation will result in concentration of zoonotic agents present in the red blood cell fraction; however a number of potential zoonoses are not present in Australia, due to being subjected to national surveillance and control measures. Other parasites, such as nematode or trematode parasites and skin infections such as ringworm, would not be transmissible through blood.

Q fever and babesiosis are caused by intracellular organisms which would be concentrated in the red blood cell fraction following centrifugation. Both organisms are present in Australia at low levels, babesiosis being associated with Northern Australia, where it is transmitted between cattle via the cattle tick *Boophilus microplus* and Q fever estimated to be present in 12% of the cattle population.

Blood Components

Blood constitutes approximately 3-5% of an animal's live weight and contains 18-19% protein; red blood cells contain 34-38% protein while plasma contains 7-8% protein with the remainder composed of 91% water and 1% low molecular weight compounds and salts. The plasma protein fraction can be separated into albumin, α, β and γ-globulins, and fibrinogen.

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.

Physical Functionality of Blood Components

Blood plasma has been shown to exhibit 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. Gels produced with plasma have been demonstrated to be stronger than those produced with serum and is due to the influence of fibrinogen during heat-induced gelation, with pH7 reported as the optimum pH for gel formation for bovine plasma.

Bovine blood plasma has been reported to have maximum hydrophobicity at pH 3, while maximum emulsifying activity at pH 7. Solubility and emulsion 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 emulsion activity at pH 5.

Bovine serum albumin (BSA) appears to be the dominating gel-forming protein in 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°C to 74°C, while increasing the free fatty acid concentration by 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. Foaming capacity was similar to egg white, but foam stability was lower and emulsification properties of blood plasma and component proteins were better than egg white.

SDP has been reported to have high gel strength capacity above 90°C with peak gel strength at 121°C, while water holding capacity of the gel was found to be stable above 80°C. When compared with other binding agents, SDP was found to be soluble, with better gelling properties than egg albumin, wheat gluten, and porcine protein but less than carrageenan.

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

Storage and microbial stability of blood

Hygienic collection of blood is vital to minimise the initial level of spoilage organisms present. Refrigeration is the most effective means of preserving blood, although food additives such as the curing agent preservatives, nitrites and nitrates can be used to extend the storage life.

1.2 Stage 2 – Production, Stabilisation and Use of Liquid Plasma

Stage 2 of the project investigated technologies to microbiologically stabilise blood for storage and transport with minimal refrigeration, investigated technologies to separate plasma from red blood cells, compare the functional properties of ssLP with SDP, and determine the proposed separation technology.

The growth of food borne bacteria is suppressed by the use of hurdle technologies such as reduction in water activity, addition of preservative agents, acidic environments and temperature control. Preliminary trials with blood and plasma indicated that the water activity was significantly reduced due to the addition of a preservative and the shelf life was extended.

Laboratory experiments showed that whole blood could be stored at <25°C for several days dependent upon the additives used to reduce the water activity. Of the different plasma separation technologies examined, one was deemed the most cost effective. Other methods were not possible due to severe fouling of the separation device or the need to remove the preservatives added to stabilise the blood prior to separation. The functionalities of the fresh and frozen plasma samples were examined. Fresh and frozen plasma samples demonstrated improved functionalities than SDP.

1.3 Stage 3 – Lab scale Proof of Concept

Stage 3 of the project investigated technological options to concentrate and microbiologically stabilise plasma and comparison of the functional properties of concentrated plasma with SDP. Laboratory experiments demonstrated that plasma could be concentrated using membrane technologies, to achieve protein concentrations of up to 25% in the concentrate. This was achieved by concentrating the plasma by a factor of 4 by volume. Functionality tests indicated that concentrated plasma was better than SDP.

Experimental data suggested treatment of concentrated plasma with a preservative resulted in a stable product stored at elevated temperatures for several days.

The results indicated that plasma could be concentrated to produce a product with functionality properties superior to SDP. Treatment with preservatives had a suppressant effect on the microbial growth and the experimental data suggested that concentrated plasma could be stored at elevated temperatures if the original plasma is of a good hygienic standard.

1.4 Stage 4 – Pilot Scale Production and Processing

Stage 4 of the project focused on scaling up the laboratory process to pilot scale, followed by the production of concentrated plasma for pilot scale functionality testing and laboratory scale shelf life studies. Four separate batches of plasma product were produced for this purpose. The laboratory scale shelf life trials suggested that the addition of a preservative to the concentrated plasma in conjunction with storing the plasma concentrate at a temperature

below 25°C was capable of extending shelf life by several days. The shelf life trials confirmed the need for microbiologically good quality blood and plasma as the feed source.

1.5 Stage 5 – Commercial Scale Production and Processing

1.5.1 Blood Collection

The colour of plasma had been identified as a potential issue; too much red in the plasma could negatively impact the colour of the plasma and subsequent products it was added to. By minimising the chances of RBC lysis the quality and quantity of plasma able to be used in products may be increased. In addition, in order to separate plasma from red blood cells (RBC) the blood needed to be in an uncoagulated form.

A third party supplier in conjunction with the Food Manufacturer commissioned equipment in the abattoir to collect and cool blood. In order to minimise water contamination via anticoagulant addition two concentrations of anticoagulant were tested. A further water free only sample was added to determine if a water free version could effectively inhibit coagulation. Finally a time course was conducted to identify what time frame the anticoagulant needed to be added in order to inhibit coagulation while maintaining minimal cell lysis.

It was found that over- or under- addition of anticoagulant led to an increase in haemolysis; while storage overnight demonstrated a further increase in RBC haemolysis. As a result it was clear that the lowest levels of anticoagulant used to inhibit blood coagulation should be implemented to minimise both cost and haemolysis of RBC and that the separation of the RBC and plasma should occur as quickly as possible. Water free anti-coagulant was deemed too inconsistent.

1.5.2 Blood Volumes

In order to correctly apply the anticoagulant, the average amount of blood per beast needed to be calculated. This allowed a dosing schedule for the anticoagulant to be determined to ensure there was enough anticoagulant to inhibit the coagulation process.

The total volume of blood from mammals is based on the weight of the animal and is reported to be between 5-8% of total body weight. Literature states that for cattle the total blood volume can also be dependent on the breed of cattle with variations of 5-9% between Hereford and Brahman breeds. Due to the large spread in variation between sex, breed and size of beasts it was not possible to obtain an accurate estimate of blood volume. An experiment was therefore conducted to determine the average volume of blood collected per beast and the average time on the slaughter table was designed.

A simple collection system was devised consisting of a stainless steel collection pan located under the slaughter table. The blood was transferred from the collection pan via a diaphragm pump to a 20L bucket before disposal of the blood down the normal path at the abattoir. The number of animals required to fill 20L was recorded along with the time each beast was on the slaughter table.

On average 11.2L of blood was collected per beast with an average time on the slaughter table of 49 seconds. This equated to approximately 1L of blood every 4 sec. However the

flow of blood was not consistent for the entire time present on the slaughter table. Along with the physical differences between the animals, the operator or slaughterman played a key role in the collection process. In some cases the location of the beast on the slaughter table greatly impacted the ability of blood to fall in to the collection pan which resulted in reduced collection figures.

Even though only a fraction of the theoretical blood volume of an animal could be collected (483L from 43 beasts), it was still evident that large volumes of usable blood could be obtained for the production of plasma if a suitable collection system could be implemented removing the issues identified during this study. This would not only involve further optimisation of an already known process, but changes to the abattoirs standard operating procedures and ways of working in order to obtain a fully compliant system producing high quality blood. While closed collection systems, such as the hollow knife technology, can be used to increase collection volumes and produce a cleaner source of blood, the cost associated with this process is most likely prohibitive.

1.5.3 On-Site Blood Collection

Ideally the entire process, blood collection through to processing, would be conducted at the abattoir to minimise any associated issues with transportation. However, due to limited space, the anticoagulant system was designed and installed at the abattoir, while the Research Provider conducted the separation and concentration at an off-site location.

Through literature searches and experience, a blood collection and cooling system was conceived (with major input from the third party) to be installed at the abattoir. A pump was installed to move the uncoagulated blood from the collection pan, located beneath the kill table, to the storage unit outside. During transportation of the blood to the storage pallecon (1000L bladder), the uncoagulated blood passed through a heat exchanger to reduce the temperature.

Based on the average volume of blood collected per beast (11.2L) a dosing schedule was devised to add anti-coagulant directly to the collection pan during slaughter. The collection pan held 40L of liquid; therefore when the collection pan was full the pump was turned on for a period of time calculated to drain 20L at a time.

It was shown that the collection system designed was capable of collecting uncoagulated blood. However, the quantity and quality of the blood collected limited the amount of plasma that could be obtained and further processing. The blood collection trials at the abattoir resulted in a number of learning’s and the identification of improvements that could be applied if this type of blood collection was required in the future. As blood collection is a fairly well known process, and there are companies within Australian and globally with this knowledge, no further work was undertaken on blood collection in this project.

1.5.4 Blood Processing

Single strength plasma (ssLP) was obtained and separated using a similar method as was proposed for the original trial. The plasma was put through the membrane technology. The membrane technology was able to concentrate the protein with a recovery of 96%. Following concentration the membrane technology was able to be completely recovered. The use of the membrane technology had no net benefit on the final microbial load.

During Stage IV of the project, the high bacterial load present in the starting material led to a recommendation to further investigate the potential use of technologies to reduce the microbial load of the blood, the ssLP or the cLP. A technology was evaluated to determine its ability to further reduce the microbial load of cLP. Three different configurations of the technology were evaluated. Two of the configurations were able to reduce bacterial load. However, they also retained protein. The third configuration had the opposite effect, resulting in both the bacteria and protein passing through the technology for no gain. No other technology configurations were tested; as a result it was determined that the technology configurations examined were unable to reduce the microbial load of the material. Based on the findings and the experience gained at the abattoir, it was determined that the best method to reduce microbial contamination would be at the abattoir during blood collection.

1.5.5 Plasma Stabilisation

The Research Provider clearly demonstrated the ability to use preservatives to alter the water activity (a_w) of blood and plasma to inhibit bacterial growth. Work in previous stages showed various levels of preservatives could reduce a_w, resulting in inhibition of bacterial growth for several days at various temperatures. As there was a need to reduce the concentration of preservative used, trials were undertaken to determine if lower concentrations could inhibit bacterial growth in conjunction with reduced temperature for stabilisation purposes. The results demonstrated that the commercial scale material could be stored with lower preservative levels.

1.5.6 Applications of concentrated liquid plasma

Following successful functionality studies, multiple pilot scale batches of material were produced and sent to the Food Manufacturer for trials. Multiple trials were conducted with the cLP and it was demonstrated to be suitable alternative to SDP.

1.5.7 Business models

Although not detailed in this report, a number of business models were analysed to determine the final cost of the cLP and the commercial viability of the process. Model 1 (the entire collection and processing is carried out at the abattoir) was found to be the most economical (Table 1). As multiple assumptions were made and it is recommended that independent advice and costs be obtained to determine and validate the economic viability.

Table 1: *Production costs*

	Cost ($AUD)
Estimated Concentrated Liquid Plasma Production Costs	$540 per tonne*
Estimated Production cost per head of cattle	$0.97*

**multiple assumptions were made including but not limited to: production costs based on entire process located at the abattoir, abattoir processing 1500 head/day, and 5 days/week. It is recommended that individual abattoirs obtain independent economic analysis for their particular situation*

Conclusion

The project identified the production of **concentrated liquid plasma** (cLP) that may be lower in cost than currently available **spray dried plasma** (SDP) as an opportunity for exploration. To stabilise blood for separation, multiple anti-coagulants were analysed, and one was determined to be the safest, most cost effective anticoagulant for use in material to be used for food manufacture. The concentration of anti-coagulant was optimised for the collection process designed in conjunction with the abattoir and a third party manufacturer. Pilot scale plasma concentration trials using membrane technologies successfully produced concentrated plasma with a high protein recovery. As expected, the addition of preservatives in conjunction with reduced storage temperature resulted in stabilisation of the material for a significant period of time. The functionality of the cLP samples stored over this period did not indicate any change as a result of storage temperature or the addition of preservatives. The cLP produced and stored under these conditions was successfully trialled in a variety of products. An outline of the process developed and estimated proximate analysis of the final product is shown in Figure 1 and Table 2 below.

Figure 1: *Process flow diagram for production of cLP*

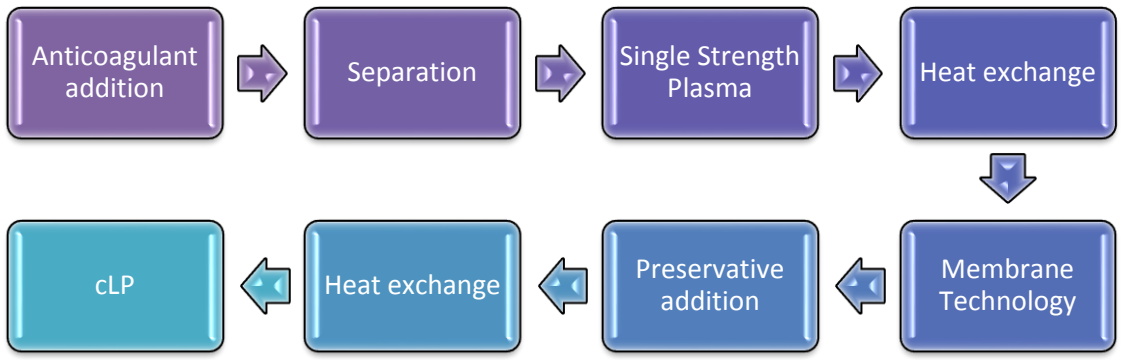

Table 2: *Proximate analysis (estimates based on trial production runs)*

	Percentage
Protein	20-25
Fat	<0.5
Ash	<6.0
Carbohydrate	1-3
Moisture	65-75