Improved plasma recovery by means of red cell crenation assisted by RSM approach

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Abstract – Blood plasma is a rich-protein liquid which has many applications in food, research, pet food and animal feed industries. However, for some applications, the haemoglobin level in plasma needs to be very low to prevent, for example, interference with other proteins, color changes or bitter/metallic taste. Plasma is obtained mainly by centrifugation, but as the processing volume increases, the haemoglobin content increases as well, and the plasma quality is considered poor. In this research a novel process, based on red cells crenation, is presented as a method to obtain low-haemoglobin content plasma, even when large volumes are centrifuged. Four factors were considered to affect the plasma quality: processing time and volume, centrifugation force, and concentration of phosphate saline buffer in plasma. Results showed that 10% v/v 10X-PBS treated-blood samples were able to generate better quality plasma than untreated samples regardless the speed, volume or time employed. Plasma obtained displayed similar functional properties as controls; even more ethyl fractionation following Cohn's method generated rich protein fraction with identical SDS-PAGE profile than controls.

Key Words – blood plasma, low-haemoglobin content, recovery of value.

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I. INTRODUCTION

Blood is one of the main co-products generated by the meat industry and while it is an excellent source of high quality proteins, it also hold high potential as a pollutant. These factors have led to increased interest in a more complete recovery and optimum utilization of this particular co-product (1). Blood is underutilized and many applications in pet food, animal feed, composting or biogas generation, while providing value, are generally low added-value (2). One of the main steps in using blood protein requires centrifugation to yield plasma and red cell fractions. The use of plasma alone overcomes the bitter and metallic taste that whole blood can impart when used as an ingredient: this also reduces the intense red color from the haemoglobin which can negatively affect product acceptance. In this sense, the quality of plasma can be defined by a low haemoglobin concentration (lower than 0.5 mg/ml). High quality plasma can be easily obtained by centrifugation when low volumes are employed; however, as the processing volumes increase, blood separation becomes more difficult, and the plasma quality is poorer.

Red cells exposed to hyperosmotic media release water to plasma, thus density difference between plasma and red cells increases. This phenomenon is call red cell crenation. The aim of this research was to obtain plasma with low haemoglobin-content when large blood volumes are processed. In this research red cell crenation was carried out by adding specific amounts of highly concentrated phosphate saline buffer (PBS) to fresh blood. A response surface methodology approach was established in order to investigate the effect of the main processing parameters: processing volume and time, centrifugation force and concentration of PBS buffer added to the blood.

II. MATERIALS AND METHODS

Blood was supplied by Irish beef abattoirs. Blood was treated with an anticoagulant and processed within 24 hours after reception and no more than 48h after collection.. In order to determine the effect of the four factors a Box-Borkenhagen experiment was designed. Each one of the factors has three levels and the ranges were established based on previous experiments. Factors studied were: processing time (5 to 15 minutes), processing volume (60 to 340 mL per centrifuge tube), centrifugation speed (3000 to 10000 g) and the concentration of the PBS buffer added (1% to 10% w/v, 1% was considered as untreated sample since no crenation or haemolysis was detected). The design consisted of 27 runs, each one carried out by duplicate. Two different batches from each one of two suppliers were processed following the experimental design runs. The PBS buffer was added slowly under continuous gentle stirring to a final 1/9 buffer /blood ratio. Blood was then centrifuged according to the experimental design. After centrifugation plasma was decanted manually and plasma volume was recorded, haemoglobin concentration calculated spectrophotometrically, and protein concentration determined by Dumas method using a LECO system. Red cells crenation was confirmed by optical microscopy.

III. RESULTS AND DISCUSSION

A statistical analysis of the results was performed in order to determine the effect of each one of the factors on the haemoglobin content, protein content and volume of plasma recovered. The quadratic models were significant ($P<0.0001$) and the coefficients of determination (R^2) of were satisfactory. The predicted R^2 were in reasonable agreement of the adjusted R^2 in all the models. The analysis of error indicated that the lack of fit tests were not

significant (P>0.05) confirming the validity of the models. The parameters that are significant for the model are summarized in Table 1. It was observed, that PBS has a positive effect on the volume of plasma recovered (achieving recovery yields of 75%, compared to a 60% yield of the control). Haemoglobin concentration in plasma was remarkably affected by PBS concentration. For instance, after centrifugation of 340ml of blood at 10,000g, the haemoglobin content was 2.1±0.1 mg/mL, whereas addition of 10%v/v of 10X PBS gave a 10-fold reduction in haemoglobin content (0.21±0.01 mg/mL). As expected, protein concentration in plasma is lower, due to the water released from red cells. Nevertheless, it was observed that the total amount of protein recovered per volume of blood processed increased as the concentration of PBS increases. For example the optimal model compared to the control recovered 3.42±0.08 and 3.78±0.02 g/100mL of blood, respectively. This may be due to the fact that higher red cell compaction levels are reached, so less plasma remains in the interstitial gaps between cells in the pellet.

Table 1: significant factors ($p<0.05$) affecting the responses studied. A: volume (mL); B: time (min); C: centrifugation speed (g) and D: concentration of PBS buffer added (%w/v). R^2, R^2adj, R^2 predicted and lack of fitness are also presented.

	Hb content	**Plasma yield**	**Protein concentration in plasma**
Significant factors	A, D, A^2, D^2	A, B, C, D,B^2,C^2,D^2, AB, BC	B, C, D, D^2
R^2	74.72	96.06	85.28
R^2 adjusted	61.96	89.30	77.38
R^2 predicted	70.12	93.97	82.60
Lack of fit	0.431	0.055	0.471

Models were validated and optimum conditions for scaling-up were selected: 10 min of processing time, and 10% v/v of 10x PBS buffer. Trials using 800 mL were carried out and the plasma obtained was found to have 0.20±0.03 mg/mL of haemoglobin, 69±1% plasma yield, 5.10±0.02% protein content and 3.51g of protein/100 mL of blood. This plasma was further spray dried, the techno-functional properties (solubility, emulsifying, gelling and water and oil holding capacity) and SDS-PAGE profile were tested. No differences were found when compared to values reported on bibliography (3). Even more, ethyl fractionation was conducted and the profile of fractions was identical than those reported in literature.

IV. CONCLUSION

It was shown that with a simple pretreatment plasma can successfully obtained with very low haemoglobin content under many different processing conditions. Main results showed that blood with a 10% v/v addition of 10X PBS buffer yielded plasma of very low haemoglobin-content (<0.5 mg/mL), regardless of volume or centrifugation speed, if at least 10 minutes of processing are employed. Besides, highest amounts of protein can be recovered. Furthermore, it was shown that scaling-up the processing volume (X 3), the plasma haemoglobin remained lower than 0.5 mg/mL. This outcome provides a good basis for carrying out further research to obtain high quality plasma at volumes of more relevance to industry using continuous centrifuges.

ACKNOWLEDGEMENTS

"This work forms part of the ReValueProtein Research Project (Grant Award No. 11/F/043) supported by the Department of Agriculture, Food and the Marine (DAFM) under the National Development Plan2007–2013 funded by the Irish Government."

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