Separation of porcine blood. Use of the red cell fraction in meat products and preparation of blood platelets.

E. SLINDE and G. BRØNSTAD

MATFORSK, Norwegian Food Research Institute, N-1430 Ås, and BioSmia A/S, N-7760 Snåsa, Norway.

SUMMARY: By the use of blood it is possible to increase the amount of heme iron in minced meat products and to optimize the red colour. Hemoglobin and myoglobin have been quantified by high-performance liquid chromatography using a Bio-Gel TSK phenyl-5PW column.

Blood platelets contain a number of growth factors that stimulate cells in tissue culture and are effective in wound healing. By using biocompatible materials and continuous flow centrifugation kilogram quantities of porcine blood platelets have been prepared. Thrombin releasate of the platelets stimulates cells in tissue culture.

INTRODUCTION: Animal blood represents both a nutritional source as well as a source for biotechnology products. The present study concerns use of animal blood as a colour and heme ingredient in meat emulsion products, and a source for production of blood platelets that can be used for wound healing.

Iron deficiency is by far the most common nutritional disorder and cause of anemia in industrialized countries. As the availability of heme iron is recorded to be 3-10 times that of non-heme iron, it should be of interest to increase the amount of heme iron in our diets. Blood may be added to meat emulsion products in small amounts (SLINDE and MARTENS, 1982; MIELNIK and SLINDE 1983; OELLINGRATH and SLINDE 1985, 1988). Quantitative determination of myoglobin and hemoglobin in the raw materials, and hemoglobin in the blood is essential for colour optimalization of a recipe. Such a method has recently been published by OELLINGRATH et al. (1990). In the present study it is shown that by using this method to measure the amount of hemoglobin and myoglobin in standardized raw materials and in blood it should be possible to calculate the amount of pigment in a recipe.

It has been shown that platelet-derived wound healing factors from autologous platelets stimulate repair of chronic wounds up to 100 per cent (KNIGHTON et al. 1986, 1990). It has also been shown that releasate from heterologous platelets can be used with similar results (CARTER et al. 1988; KSANDER et al. 1990). Since Norwegian pig production is characterized by a unique health situation, porcine blood platelets may be a suitable source for production of releasate. We show here that kilogram quantities of porcine blood platelets may be produced by continuous flow centrifugation, and that a thrombin releasate from such platelets stimulates growth of cells in tissue culture.

MATERIALS AND METHODS: Determination of myoglobin and hemoglobin was performed as described by OELLINGRATH and SLINDE (1985), and OELLINGRATH et al. (1990).

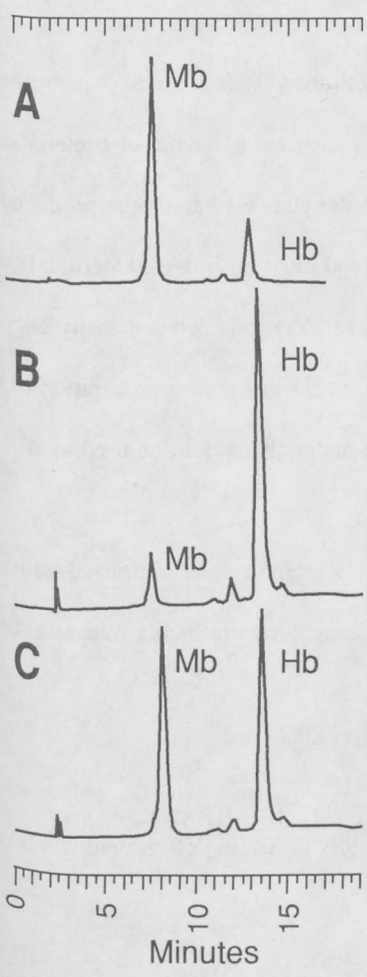
Porcine blood platelets were prepared as follows. The blood were drawn from the pigs, citrated, and left in fifty liter tanks overnight at 4 °C. The plasma was removed and centrifuged in a Beckman centrifuge using a JCF-Z zonal rotor with the continuous-flow small zonal core. The small amount of blood cells in the plasma was removed by centrifugation at 4200 rpm

and the supernatant was centrifuged at 8000 rpm to collect the blood platelets. From 250 l whole blood, approx. 450 g wet weight of white platelets were obtained. Ten gram freshly prepared platelets were resuspended in 500 ml buffer (50 mM HEPES (*N*-2-hydroxy-ethylpiperazine-*N*'-2-ethanesulphonic acid), 100 mM NaCl, 6 mM KCl and 3mM glucose, pH 7.4). One unit thrombin ("Thrombinar", Johnson Medical Industries Inc., St.Louis, USA) per ml was added and the suspension was incubated at 20 °C for 10 min. The platelet suspension was centrifuged at 20000 g for 15 min. The supernatant was pipetted off and sterile filtered through a 0.2 µ filter. Stimulation of thymidine incorporation was measured on C3H 10 T/2 fibroblasts in culture.

Total number of bacteria was determined on plate count agar (PCA, Difco). Protein was determined according to LOWRY et al. (1951).

RESULTS and DISCUSSION:

Chromatograms of drip from pork meat and pig blood are shown in Figure 1 A and B. Both hemoglobin and myoglobin can be quantified in the two samples. In Figure 1 C a chromatogram where myoglobin and hemoglobin have equal absorption at 420 nm is shown. This chromatogram was obtained by mixing a calculated amount of the samples used to obtain Figure 1 A and B. OELLINGRATH et al. (1990) have shown that a quantitative determination of myoglobin and hemoglobin is possible in meat minces. In a heated product the contribution to colour from hemoglobin and myoglobin is reflected by the total heme concentration. By the use of the present approach it is therefore possible to calculate the amount of heme iron or

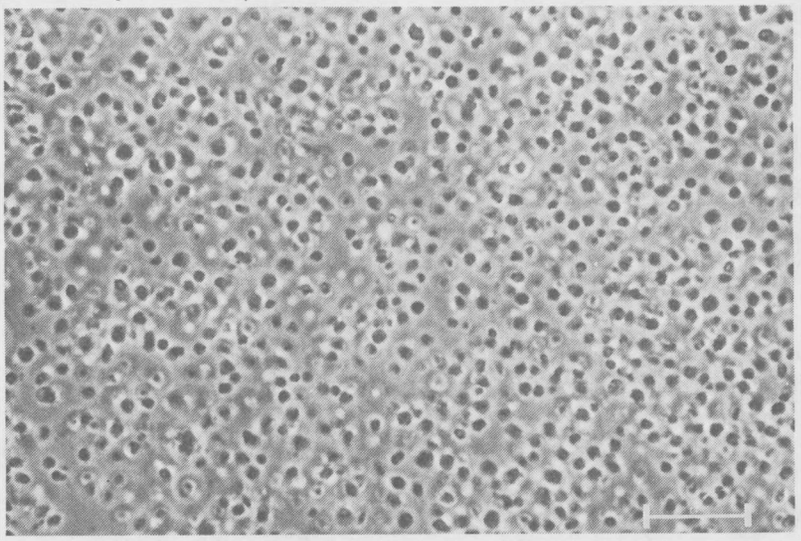


final product based on the amount of pigment in the raw materials. Animal blood may therefore be added as a contributor to colour or as a heme source.

Chromatograms of drip from pork meat and pig blood are shown in Figure 1A and B. Both hemoglobin and myoglobin can be quantified in the two samples. In Figure 1C a chromatogram where myoglobin and hemoglobin have equal absorption at 420 nm is shown. This chromatogram was obtained by mixing a calculated amount of the samples used to obtain Figure 1 A and B. OELLINGRATH et al. (1990) have shown that a quantitative determination of myoglobin and hemoglobin is possible in meat minces. In a heated product the contribution to colour from hemoglobin and myoglobin is reflected by the total heme concentration. By the use of the present approach it is therefore possible to calculate the amount of heme iron or colour in the final product based on the amount of pigment in the raw materials. Animal blood may therefore be added as a contributor to colour or as a heme source.

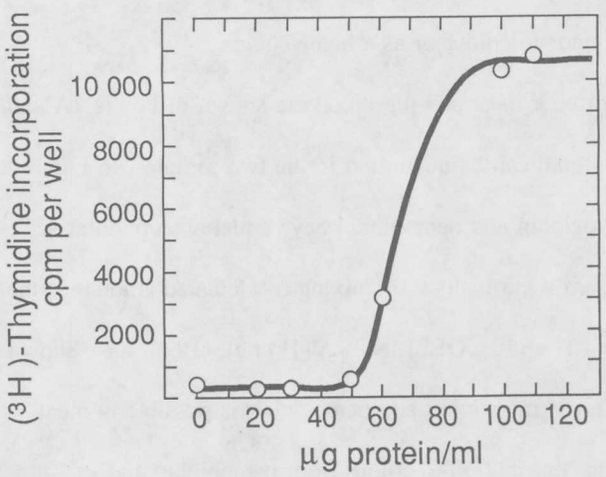
Figure 1: Separation of hemoglobin (Hb) and myoglobin (Mb) on a Bio-Gel TSK Phenyl-5-PW hydrophobic column measured at 420 nm. (A) Drip from thawed pork meat, (B) pig blood and (C) a mixture of blood and drip giving an equal amount of hemoglobin and myoglobin colour when measured at 420 nm.

Figure 2: Light microscopic picture (830 x) of porcine blood platelets. The bar represents 20 μm.



The light microscopic picture in Figure 2 shows the isolated blood platelets. The preparation is essentially free of other cellular particles from the blood. After treatment of the platelets with thrombin, the solution was centrifuged and filtered and a releasate containing 950 μg protein per ml was obtained. The proliferative activity of the releasate was quantified by measuring the (^{3}H) thymidine incorporation into DNA of C3H 10 T/2 fibroblasts in culture (Figure 3). The cells were grown to confluency in 24-well micro dishes in the presence of 10 % fetal calf serum. Two days after having reached confluency, the spent medium was replaced with fresh medium without fetal calf serum. After 17 hours (^{3}H)-thymidine was added for 4 hours, and TCA (trichloroacaetic acid) precipitable radioactivity was measured in a liquid scintillation counter. When ten per cent fetal calf serum was used the incorporation was found to be 6300 cpm/per well. The high proliferative activity of the releasate makes its use as a wound healing ingredient possible. The isolated platelets have a bacterial count of 10^4 to 10^5 per gram wet weight. At ordinary slaughter conditions we have found that the bacterial count of the blood is approx. 400/ml. Through the purification process the bacteria are concentrated together with the platelets which explain the high number found. The releasate is devoid of bacteria since this is sterile filtered. If the platelets have to be produced at sterile conditions the blood must be collected sterile. The physiological responses of blood platelets are easily elicited (LAPETINA, 1990). To avoid this during preparation of the platelets, biocompatible materials have to be used at all stages.

Figure 3: Stimulation of (^{3}H)-thymidine incorporation into C3H 10 T/2 fibroblasts as a function of added thrombin blood platelet releasate.



CONCLUSIONS: By determining hemoglobin and myoglobin in raw materials and blood, a recipe can be optimized with regard to colour or heme-iron content. Releasate of porcine blood platelets stimulate fibroblast growth. Such a releasate may be used in wound healing products.

REFERENCES:

CARTER D.M., BALIN, A.K., GOTTLIEB, A.B., EISINGER, M., LIN, A., PRATT, L., SHERBANY, A. and CALDWELL, D.(1988): Clinical experience with crude preparations of growth factors in healing of chronic wounds in human subjects. Prog. Clin. Biol. Res. 266: 303-317.

KNIGHTON. D.R., CIRESI. K., FIEGEL, V.D., SCHUMERTH, S., BUTLER, E. and CERRA, F. (1990): Stimulation of repair in chronic, nonhealing, cutaneous ulcers using platelet-derived wound healing formula. Surg. Gyn. Obst. 170: 56-60.

KNIGHTON D.R., FIEGEL, V.D., AUSTIN, L.L., CIRESI, K.F., BUTLER, E.L. (1986): Classification and treatment of chronic nonhealing wounds. Successful treatment with autologous platelet-derived wound healing factors (PDWHF). Ann. Surg. 204: 322-330.

KSANDER, G.A., SAWAMURA, S.J., OGAWA, Y., SUNDSMO, J. and MCPHERSON, J.M. (1990): The effect of platelet releasate on wound healing in animal models. J. Am. Acad. Dermatol. 22: 781-791.

LAPETINA, E.G. (1990): The signal transduction induced by thrombin in human platelets. FEBS Lett. 268: 400-404.

LOWRY. O.H., ROSEBROUGH, N.J., FARR, A.L. and RANDALL, R.J. (1951): Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

MIELNIK, J. and SLINDE, E. (1983) Sausage colour measured by integrating sphere reflectance spectrophotometry when whole blood or blood cured by nitrite is added to sausages. J. Food Sci. 48: 1723-1725.

OELLIGRATH, I.M., IVERSEN, A. and SKREDE, G. (1990): Quantitative determination of myoglobin and haemoglobin in beef by high-performance liquid chromatography. Meat Sci. 28: 313-320.

OELLINGRATH, I.M. and SLINDE, E. (1985): Color, pigment and iron content of meat loaves with blood, blood emulsion, or mechanically deboned meat added. J. Food Sci. 50: 1551-1555.

OELLINGRATH, I.M. and SLINDE, E. (1988): Sensory evaluation of rancidity and off-flavour in frozen stored meat loaves fortified with blood. J. Food Sci. 53: 967-968.

SLINDE, E. and MARTENS, M. (1982): Changes in sensory properties of sausages when small amounts of blood replace meat. J. Sci. Food Agric. 33: 760-762.