D52

STUDIES ON EFFICIENT UTILIZATION OF PORCINE BLOOD AS FUNCTIONAL FOOD AND FOR PHARMACEUTICAL USE

KAZUHISA HONDA, MAKI KAWAGUCHI, SHIGEAKI WATANABE, MASAHIRO NUMATA AND TOYOO NAKAMURA
ITOHAM FOODS INC., 1-2 Kubogaoka, Moriya, Kitasouma, Ibaraki Pref., 302-01, Japan
Keywords: blood, hemoglobin, heme, hemin, by-product, adipocyte differentiation

Background and objective

Animal by-products with economic value are obtained from an animal during slaughter and processing. Although accounting for only a small fraction of the current live animal value, they are of considerable economic importance to the entire livestock and meat industry. In addition to the monetary value that is derived from the processed by-product, the conversion of inedible parts of the animal into useful products performs a very important fraction from a sanitary standpoint. Because all inedible parts, unless processed or disposed of in a proper manner, would accumulate and decompose, causing undesirable conditions in the surrounding environment.
Very large volumes of blood are produced as a result of slaughtering. Although about ten percent of the total protein content of an animal can be accounted for by blood protein, most of this protein is underutilized in the sense that it is dried for use in animal feed or fertilizers. In Japan, there is little use for blood as a component of blood sausages or seasoning. Then, most blood has been wasted at a heavy cost, although it had a highly nutritious potential. This investigation was carried out for efficient utilization of porcine blood which is the main animal by-product in Japan.

Methods

Functional food materials

Fresh porcine blood was obtained from a slaughter plant (Kagoshima, Japan) and 0.5% sodium citrate was added as an anticoagulant. The blood was separated to plasma and blood cell fraction by centrifugation. The plasma was concentrated by ultrafiltration and frozen before use. On the other hand, the blood cell fraction which almost completely consisted of red blood cells was dissolved in water and acidified. The solution was digested by acid protease and centrifuged to separate the heme rich precipitant and protein rich supernatant. The precipitant was washed in water and centrifuged. The obtained heme-iron concentrate as a precipitant was resuspended in water and spray-dried.
The protein rich supernatant was neutralized and filtered. The filtrate was spray-dried to obtain the globin digest as a white powder.

Pharmaceuticals

The heme-iron concentrate was resusupended in water and acidified. The suspension was boiled with detergent and cooled to room temperature. This suspension was centrifuged to collect the appeared hemin particles as precipitant. The precipitant was resuspended in water and spray-dried.
Globin digest was dissolved in water and ion exchange resin added to remove ionic peptides. The supernatant was fractionated by reversed-phase chromatography to purify ADIP (Adipocyte Differentiation Inhibiting Peptide) and lyophilized.

Analytical procedures

The protein content was calculated by a micro Kjeldahl method modified by deduction of hemin derived nitrogen. The hemin content was quantitated by the pyridine hemochrome method. The iron content was determined by the o-phenanthroline method after ashing. Inhibition of adipocyte differentiation was expressed by inhibition of GPDH (Glycerol-3-phosphate dehydrogenase) activity of Swiss mouse 3T3-L1 cells. The method of cell culture was described by Green et al[1]. Statistical analysis was performed by Student's t-test.

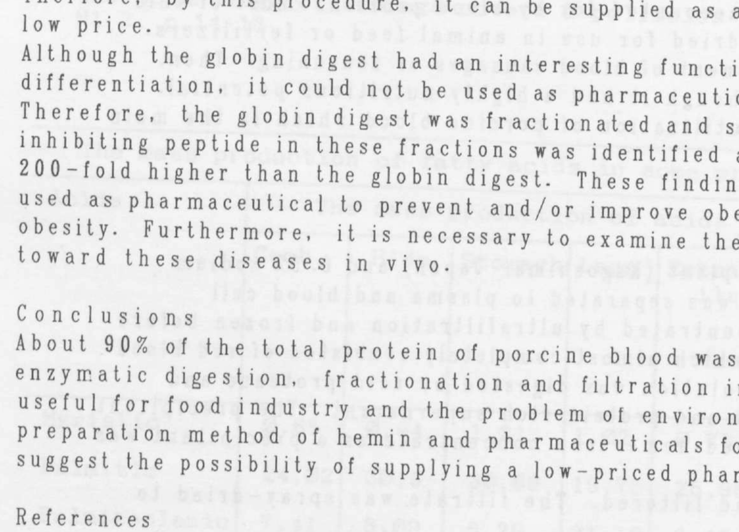
Results and discussion

Scheme 1 shows the preparation method of functional food materials and Table 1 the yield of protein. From these preparations, about 90% of the blood protein could be utilized as edible proteins.
Concentrated plasma can be used as a binding agent or emulsifier of processed meat products and indicated the same effect compared to other proteinaceous additives. Furthermore, it was more useful compared with powder, because it did not need to be resuspended and could be used as ice.
The heme-iron concentrate can be used as functional food material to supply iron. Heme-iron is considered to be absorbed as the intact iron porphyrin complex and the iron to be released

inside the intestinal mucosal cell[2]. The bioavailability was five-fold higher than that of nonheme-iron[3]. Furthermore, nonheme-iron is considered to inhibit the absorption by many factors in human food[4]. In this study, heme-iron was concentrated above 1%. The concentration was five-fold higher than that in the dried blood (0.2%). These findings suggest that the addition of a small amount of heme-iron concentrate to food can correct iron deficiency.
The globin digest is the mixture of peptides obtained from hemoglobin by enzymatic digestion. The addition of globin digest to culture medium inhibited differentiation to adipocytes on Swiss mouse 3T3-L1 cells. This effect was dose dependent. Furthermore, the inhibitory effect on adipocyte differentiation was also observed in vivo. Therefore, the addition of globin digest to food may prevent obesity.
To utilize these materials as pharmaceuticals, hemin and ADIP were purified. Hemin is used as pharmaceutical material for hepatic diseases. The hemin content of heme-iron concentrate was about 10% and the others were insoluble peptides. These peptides could be solved by heating with detergent and excluded by centrifugation. By this procedure, hemin was purified above 90% and the yield was about 90% from the heme-iron concentrate. The present method is very efficient and low cost, because it does not require any organic solvent and many acids. Therfore, by this procedure, it can be supplied as a pharmaceutical for hepatic diseases of a low price.
Although the globin digest had an interesting function as inhibitor of the adipocyte differentiation, it could not be used as pharmaceuticals, because it was a mixture of peptides. Therefore, the globin digest was fractionated and screened by the inhibitory effect. The most inhibiting peptide in these fractions was identified and named ADIP. The inhibitory effect was 200-fold higher than the globin digest. These findings suggest that ADIP had a potential to be used as pharmaceutical to prevent and/or improve obesity and other diseases derived from obesity. Furthermore, it is necessary to examine the contribution of ADIP and its analogue toward these diseases in vivo.

Conclusions
About 90% of the total protein of porcine blood was utilized as functional food materials by enzymatic digestion, fractionation and filtration including concentration. The product was useful for food industry and the problem of environmental pollution. Furthermore, a useful preparation method of hemin and pharmaceuticals for obesity was discovered. These findings suggest the possibility of supplying a low-priced pharmaceutical for human health.

References
1)Green, H. et al, Cell, 7, 105, 1976.
2)Hallberg, L. et al, Acta Med. Scand., 181, 335, 1967.
3)Hallberg, L. et al, Scand. J. Gastroent, 14, 769, 1979.
4)Morris, E. R. et al, J. Nutr. 106, 753, 1976.



Scheme 1 Preparation of functional food materials from porcine blood.

Food materials	Rate(%)
Blood	100
Concentrated plasma	23.1
Heme-iron concentrate	25.7
Globin digest	38.5
Waste protein	12.7

Table 1 Protein distribution of blood protein to functional food materials.