Decolouration of slaughterhouse blood by treatment with hydrogen peroxide

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Erythrocyte or haemoglobin fraction from blood is a potential protein source which is not yet explored to any appreciable extent: application of this material is hampered by its strong colour. Various methods for decolouration of haemoglobin have been described in the literature, and one method, oxidation with hydrogen peroxide, seems to be a very simple one.

The objectives of the work were: to check the optimal conditions for the oxidation process, to evaluate the potentials for scaling up, and to evaluate the properties of the decoloured protein.

The conditions (pH, temperature, amount of hydrogen peroxide) for decolouration of the erythrocyte ("thick blood") fraction of blood were studied. The decoloured proteinaceous material was analysed and its technological properties evaluated.

A yellowish proteinaceous material can be obtained by oxidation of the erythrocyte (haemoglobin) fraction of blood with hydrogen peroxide. The conditions for the process are very simple and allow large-scale production. The product can be obtained as a wet press cake (50% water) or as dry beads. The yield, in terms of protein recovery, is 90%. The dry material consists of 99% protein and still contains all the (haemoglobin) ions (0.28%).

The dry decoloured protein can be incorporated into cooked comminuted meat products at a level of 1 to 1.5%, thus yielding 5 to 10% of the total protein, without affecting texture, taste and colour adversely.

The net protein utilisation (NPU) in rats of the decoloured "haemoglobin" was similar to that of the original (heat denatured) "thick blood". Before the decoloured material can actually be applied in meat products, a long term feeding test to reveal any toxicologic effects, should be carried out.

Entfärbung von Schlachthausblut durch Behandlung mit Wasserstoffperoxid

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Die Erythocyten- oder Hämoglobin-Fraktion aus Blut stellt eine potentielle Proteinquelle dar, die noch nicht in beachtlichem Masse untersucht worden ist, weil die dunkle Färbung einer Verwendung noch im Wege steht. Unterschiedliche Methoden zur Entfärbung des Hämoglobins sind im Schrifttum beschirieben worden, und eine davon, die Oxidierung mittels Wasserstoffperoxid scheint am einfachtsten durchführbar zu sein.

Unsere Untersuchungen hatten zum Ziel: Festsetzung der optimalen Bedingungen für den Oxidationsvorgang, Auswertung der Möglichkeiten zur Vergrösserung des Verfahrens im technischen Massstab und Bestimmung der Eigenschaften des entfärbten Proteins.

Es werden die Bedingungen für die Entfärbung des Erythrocyten-Fraktion ("dickes Blut"), wie pH-Wert, Temperatur, erforderte Menge Wasserstoffperoxid, beschrieben. Das entfärbte, eiweisshaltige Material wurde analysiert und dessen technologischen Eigenschaften ausgewertet.

Oxidation der Erythrocyten-Fraktion (Hämoglobin) des Blutes durch Wasserstoffperoxid ergibt ein gelbliches, eiweisshaltiges Material. Die Verfahrensbedingungen sind sehr einfach und erlauben eine Vergrösserung im technischen Massstab. Das Produkt kann erhalten werden in Form eines nassen Presskuchens (50% Wasser) oder als trockene Kügelchen. Die Ausbeute, ausgedruckt als Prozentsatz des rückgewonnenen Proteins, beträgt 90%. Das Trockenprodukt enthält neben 99% Protein auch noch das Ausgangs(Hämoglobin)ion (0,28%).

Das trockene, entfärbte Protein kann in Mengen von 1-1,5% gekochten und zerkleinerten Fleischprodukten zugegeben werden und liefert dann 5-10% des Gesamtproteins ohne Beeinträchtigung der Textur, des Geschmacks oder der Farbe.

Die Netto-Protein-Verwertung (NPU) des entfärbten Hämoglobins in Ratten war identisch mit dem des originellen (hitzedenaturierten) "dicken Blutes". Bevor jedoch das entfärbte Produkt angewandt werden kann, sind noch langfristige Fütterungsversuche zum Nachweis etwaiger toxicologischer Wirkungen erfordert.

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Décoloration du sang d'abattage par traitement avec de l'eau oxygénê

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La fraction érythrocytique ou hémoglobinique du sang constitue une source potentielle de protéine qui n'a pas encore été étudiée profondément du fait que l'application de cette matière est gênée par sa couleur intense. Diverses méthodes destinées à la décoloration de hémoglobine ont été décrites dans la littérature, et une méthode, notamment l'oxydation à l'eau oxygénée, apparaît particulièrement simple.

Les objectifs du travail ont été d'établir les conditions optimales pour le processus d'oxydation, d'évaluer les potentialités d'une application plus étendue, et d'apprécier les propriétés de la protéine décolorée.

Les conditions (pH, température, quantité d'eau oxygénée) favorables à la décoloration de la fraction érythrocytique ("sang épais") du sang ont été étudiées. La matière protéique décolorée a fait l'objet d'une analyse et ses propriétés technologiques ont été évaluées.

Une substance protéique jaunâtre peut être obtenue par oxydation de la fraction érythrocytique (hémoglobinique) du sang à l'aide d'eau oxygénée. Les conditions valables pour le processus sont très simples et permettent une production sur grande échelle. Le produit peut s'obtenir en tourteau de pression à l'état humide (50% d'eau) ou bien en perles sèches. Le rendement, en termes de récupération de la protéine, s'élève à 90%. La matière sèche est composée de 99% de protéine et contient encore tous les ions (hémoglobine) (0,28%).

La protéine sèche décolorée est susceptible d'être incorporée à un taux de 1-1,5% dans les produits de viande broyée cuite, donnant ainsi un rendement de 5-10% de la protéine totale, sans que la texture, le goût et la couleur subissent une influence défavorable.
L'utilisation globale de protéine (UGP) chez des rats, de l'hémoglobine décolorée était pareille à celle du "sang épais" (dénaturé à la chaleur) original. Avant que la matière ne puisse être employée effectivement dans les produits de viande, un essai d'alimentation à longue terme, propre à mettre en évidence tout effet toxicologique, doit être entrepris.

Обесцвечивание крови из боен путем обработки перекисью водорода

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Эритроцитовая (гемоглобиновая) фракция крови является пока мало изученным потенциальным источником белка, но интенсивная окраска материала препятствует его применению.

В литературе описаны различные способы обесцвечивания гемоглобина; один из них, окисление перекисью водорода, оказывается очень простым. Целью работы было изыскание оптимальных условий процесса окисления, оценка возможностей расширения масштабов процесса и изучение свойств обесцвеченного белка.

Исследованы условия обесцвечивания (pH, температура, количество H_2O_2) концентрированной эритроцитовой фракции крови. Проанализирован обесцвеченный белковый материал и определены его технологические свойства.

Представляется возможным получить белковый материал желтоватого цвета окислением эритроцитовой фракции крови перекисью водорода. Технологические режимы весьма несложные и позволяют организовать крупномасштабное производство. Продукт может вырабатываться в виде мокрого жмыха (с влажностью 50%) или сухих гранул. Выход, считая на полученный белок 90%. В сухом материале содержатся 99% белка и еще все (гемоглобиновые) ионы (0,28%).

Обесцвеченный белок вводят в вареные изделия из рубленого мяса в количестве I-I,5%, составляя 5-I0% общего содержания протеина, что не отрицательно влияет на текстуру, вкус и цвет продукта.
Питательность продукта по отношению к крысам была равной питательности необработанной (денатурированной в теплоте) концентрированной эритроцитовой фракции крови. До фактического ввода обесцвеченного продукта в мясные изделия необходимо проводить длительные опыты с целью выявления токсических свойств.

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Introduction

The erythrocyte fraction of animal blood, obtained after isolation/preparation of blood plasma by centrifugation, is a potential source of valuable protein. The erythrocyte fraction, called "centriblood" or "thick blood", contains about 33% protein, mainly haemoglobin. In a large slaughterhouse, slaughtering about one million pigs a year, some 290 tonnes/year of centriblood becomes available, yielding about 96 tonnes of protein. Application of this protein is seriously hampered by its deep colour; this material could only be incorporated in meat products at very low levels.

Decolouration of the haemoglobin would yield an attractive protein while its animal origin makes incorporation in meat products easily acceptable. For the decolouration of haemoglobin basically two methods are available.

The first method, described in the literature several times, involves removal of the coloured haem group from the protein by acidified acetone (1, 2, 3). This method yields an undenatured colourless protein. However, very large amounts of acetone are required and problems with residual acetone in the protein preparation may arise.

The second method involves oxidation with hydrogen peroxide. Although this method has been described in the literature (4, 5, 6), the nature of the protein obtained is unknown and the applicability of this process for centriblood is also unknown. As the oxidation process seems to be simple and the cost of decolouration would be low, we studied the oxidative decolouration of centriblood in relation to conditions and yield, and also evaluated the nature and technological properties of the protein obtained.

Experimental

Centriblood (33% protein), obtained fresh from a slaughterhouse, was treated with 3% hydrogen peroxide. The temperature was varied between 50 and 80°C, and the pH was varied from 7.1 to 5.0. Conditions allowing scale-up to a simple process were investigated. The procedure adopted was based on an article by BRAHN from 1941 (ref. 4).

Results

Reaction conditions

Rapid and proper mixing of centriblood and hydrogen peroxide is a prerequisite. To realize this, the viscous centriblood has to be diluted with about 7 volumes of water; a 3% peroxide concentration was chosen. Addition of peroxide to diluted centriblood at temperatures below 70°C causes strong and troublesome foaming. Centriblood should first be heated to 70°C. This means denaturation and flocculation of the protein. Decolouration of denatured haemoglobin by peroxide can only be completed at temperatures above 50°C. At 50°C, the reaction takes 30-60 min; at 70°C the reaction is completed within 10 minutes. Cooling down the hot (70°C) suspension of haemoglobin to 50°C before addition of peroxide, as suggested by BRAHN, is not necessary: it neither affected colour nor yield. Complete decolouration of centriblood could be obtained using 0.8 to 1.0 ml 3% peroxide per g centriblood. The peroxide should be added in one portion to the hot haemoglobin suspension.

The effect of pH of the original hot haemoglobin suspension on colour and yield of "bleached" protein is illustrated below.

pH-value		Colour of dried bleached protein	Protein yield from 30 g centriblood (g)
Before reaction	After reaction		
7.10	6.18	yellow/cream	8.67
6.80	6.00	"	8.67
6.45	5.74	"	8.67
6.05	5.38	"	8.55
5.55	5.00	brown/yellow	7.78
5.10	4.63	brown	6.76

The reaction can therefore best be performed at the original pH (7.10) of the centriblood.

Procedure for large-scale decolouration

An amount of 500 g centriblood, diluted with 3.5 l water, was heated to 70°C with vigorous stirring. With continued stirring, 50 ml of 3% hydrogen peroxide was added in one portion. Two to three minutes after addition of the peroxide, the suspended haemoglobin had become decoloured and coagulated into small beads of about 1 to 2 mm diameter. The beads could easily be collected on a filter. About 800 g of wet yellowish beads were obtained. Freeze-drying of the beads yielded 150 g protein; so 90% of the original protein was recovered.

Scale-up of the process using the conditions described above seems feasible.

Nature of the decoloured protein

The dried decoloured 'haemoglobin' was analysed and the analytical figures were compared with dried denatured haemoglobin obtained from centriblood with the same process as described above, but without peroxide treatment.

	Bleached/oxidised "haemoglobin" (%)	Denatured haemoglobin (%)
Dry matter	100	100
Protein	99	99
Ash	0.75	1.22
Iron	0.285	0.29

It appears that the "bleached haemoglobin" still contains iron. Presumably, only the porphyrin ring is opened and transformed into a bile pigment by the oxidation process.

Technological properties of decoloured haemoglobin

The decoloured material is completely insoluble in water and has a bland taste. Bleached centriblood (as freeze-dried beads) has been incorporated in a (sterilized) type of luncheon meat and in cooked sausage (Brüwurst) up to 10% replacement of the meat protein. The bleached protein has no binding properties, as could be expected, but rather behaved as an inert ingredient. The texture of the products became softer. Incorporation of bleached (yellowish-brown) "haemoglobin" resulted in a marked shift in the colour of the meat products from pink to reddish-brown and yellowish-brown. However, the acceptability of meat products in which bleached centriblood has been incorporated up to levels of 1 to 1.5%, or 5 to 10% protein replacement, would not be seriously hampered by the negative effect on texture and colour. At these levels the flavour and taste of the products are only slightly affected adversely.

Cost-price

Dried bleached centriblood would cost about DM 1.50 per kg. As processing is, in principle very simple, costs are determined to large extent by the price of the peroxide.

Toxicological saftey

Treatment of heamoglobin with hydrogen peroxide will presumably result in the formation of the bile pigment choleglobin and, very likely, in the formation of oxidized amino acids. Like in oxidized casein, methionine sulphone could well be formed (7). This compound is a growth depressant.

In a short-term feeding test (10 days) with Sprague Dawley rats, the net protein utilisation (NPU) of decolourized thick blood has been determined and was found to be similar to that of the original thick blood (which was heated to 70°C to flocculate the protein and then dried after filtration).

However, before the decolourized centriblood can be applied in meat products or other foods, its toxicological safety should be examined thoroughly by generally accepted methods. An analysis of the amino acid pattern after oxidation could precede the toxicological tests to get quick information on the type and extent of the changes that occurred.

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