Das Abfärben des Schlachttierblutes durch partielle Enzymhydrolyse

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In der vorliegenden Arbeit ist es erwiesen, dass man durch eine gesteuerte partielle enzymatische Hydrolyse der abzentrifugierten zelligen Bestandteile von Schlachttierblut mit einer Ausbeute von 60-80% des Eiweissinhalts des Ausgangsmaterials Hydrolysate herstellen kann, welche leicht mittels Aktivkohle abgefärbt werden können.

Das Enzym Alcalase® 0,6 L - ein "food-grade"-Enzym der Firma NOVO - hat die besten Ergebnisse gegeben. Die Arbeit hat teils eine Optimierung der Enzymreaktion und teils eine Optimierung des Zusammenhangs zwischen dem Hydrolysengrad, den Reaktionsbedingungen, der Nachbehandlung und dem Abfärbungsgrad des Hydrolysates umfasst.

Auf Grund des Zusammenhangs zwischen der Aktivität und der Denaturierung des Enzyms ist die Temperatur auf 55°C gewählt worden, das pH-Optimum ist etwa 8,5, die optimale Substratkonzentration 8-12% Eiweiss, und das Enzym--Substrat-Verhältnis (E:S) 2-4%. Die Reaktionszeit variiert von 50-150 Minuten infolge der Unterschiede in der Vorbehandlung, des (E:S)-Verhältnisses, sowie der Wahl von Hydrolysengrad DH (degree of hydrolysis).

Die Ausnützung dieses Hydrolysates in Fleischwaren ist zur Zeit Gegenstand weiterer Untersuchungen, insbesondere werden die Probleme eines bitteren Geschmacks sowie eine Verbesserung der funktionellen Eigenschaften untersucht.

Decolourisation of slaughter blood by partial enzymatic hydrolysis

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This work showed that a controlled partial enzymatic hydrolysis of the cell fraction centrifuged from slaughter blood gave hydrolysates which were easily decolourised by activated carbon. The yield was of the order of 60-80% of the protein content of the basis material.

The enzyme Alcalase® 0.6 L - which is a food-grade enzyme from the firm NOVO - provided the best results. The experiments have primarily been concerned with an optimisation of both the enzymatic reaction and the degree of decolourisation as a function of the degree of hydrolysis, reaction conditions and further treatment.

Based on the connection between activity and denaturation of the enzyme, a reaction temperature of 55°C was chosen. The optimal pH was about 8.5 ; the optimal substrate concentration was 8-12% protein, and the enzyme-substrate proportion (E:S) was 2-4%. The time of reaction varied from 50-150 minutes, as a function of the differences in pre-treatment, (E:S) proportion and the choice of DH (degree of hydrolysis).

The utilisation of the hydrolysate in meat products is at present the object of further experiments, particularly with respect to the problems associated with bitter taste and improvements in the functional properties of the hydrolysates.

H 5:2

Décolorisation du sang d'abats par une hydrolyse enzymatique partielle

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Dans cette étude vous voyez démontré que par une hydrolyse enzymatique partielle contrôlée des globules rouges décentrifugés du sang d'abats, il est possible de produire des hydrolysats faciles à décolorer par le charbon actif avec un rendement de 60-80% du contenu de protéines de la substance mère.

L'enzyme d'Alcalase® 0,6 L - qui est un enzyme de "food-grade" de la firme NOVO - a donné les meilleurs résultats. L'étude a compris partiellement une optimalisation de la réaction d'enzyme partiellement une optimalisation des relations entre le degré d'hydrolyse, les conditions de réaction, le traitement complémentaire et le degré de décolorisation de l'hydrolysat.

La température a été choisie, selon les rapports entre l'activité et la dénaturation de l'enzyme, à 55°C, l'optimum de pH est environ 8,5, la concentration optimale de substrate 8-12% de protéine, la relation d'enzyme de substrate (E:S) 2-4%. Le temps de réaction est de 50-150 minutes variant à cause des différences du traitement initial, des relations (E:S) et du choix de degré d'hydrolyse DH (degree of hydrolysis).

L'exploitation de cet hydrolysat en produits carnés est pour le moment l'objet des recherches supplémentaires, spécialement on examine les problèmes concernant le goût amer et l'amélioration des qualités fonctionelles.

Выцветание крови убойных животных с помощью частичного ферментативного гидролиза.

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В данной работе показано что частичным регулируемым ферментативным гидролизом части клетки крови убойных животных, которую отделяли центрофугированием, можно изготовлять гидролизат, который легко можно выцветать активным углем.

Фермент Alcalase® 0,6 L, это фермент фирмы Ново, годный для применения в пищевых продуктах, который дает лучшие результаты. Работа частично касалась улучшения реакции фермента, частично улучшения связи между степенью гидролиза, условием реакции, дополнительной обработки и степени выцветания гидролизата.

Температуру из-за связи между активностью и денатурацией брали в 55°C, самый подходящий pH прибл. 8,5, наилучшая концентрация субстрата 8-12% протеина, отношение фермент-субстрат /E:S/ 2-4%. Время реакции от 50 - 150 мин., оно изменяется из-за разницы в предварительной обработке /Ф-С/ отношения а также выбора степени гидролиза.

Использование этого гидролизата в мясных продуктах в данное время является предметом дальнейших исследований, в особенности исследуют проблемы отн. горького вкуса и улучшения функциональных свойств.

Decolourisation of slaughter blood by partial enzymatic hydrolysis

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Blood has for years been a by-product in the meat industry and is mainly used as a base for feeding stuffs and only to a smaller degree for human consumption.

In order to utilize the protein content in the blood for human consumption, particularly in a world with a protein deficiency, numerous methods for treating blood have been proposed to overcome the various inconveniences associated with blood proteins, of which inconveniences dark colourisation of products containing haemoglobine is the essential one, the reason why we have concentrated our efforts on eliminating this colour in particular.

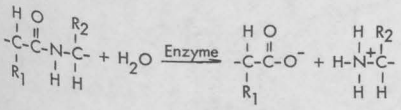
By centrifugation it is possible to separate blood into a plasma fraction (60% v/v) containing one third of the total blood proteins and a red cell fraction of 40% v/v containing the remaining two thirds of protein. The plasma proteins which have excellent functional properties may be used in a wide variety of meat products, whereas the cell fraction, due to its dark red colour that darkens the final product up to an undesirable degree, even when added in minute amounts, has not yet found sufficient fields of application. For that reason several methods have been proposed in order to remove the haem part, the colouring agent, from the haemoglobine protein, which makes up about 90% of the total cell fraction proteins. Methods involving large amounts of organic solvents based on scaling up known laboratory procedures for isolating porphyrins from chromo-proteins have been proposed (Dill et al 1) and functionality of the prepared globines has been reported (Tybor et al 2, Hermansson et al 3). However, the use of large amounts of organic solvents seems to be prohibitive in the preparation of proteins for human consumption.

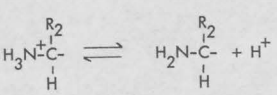
Accordingly, also other methods have been tried, some involving camouflage (ref. 4), others involving various kinds of hydrolysis in complicated systems for immobilizing enzymes and separating uncoloured peptide fractions from haemoglobine simultaneously being hydrolyzed (ref 5).

The present paper introduces a method for controlled hydrolysis of the red cell fraction followed by removal of colour by treatment with activated carbon.

Calculation

The enzyme reaction takes place according to the following scheme (a) where (b) shows the dissociation of the amino group at pH about 6.5

a 

b 

The pH-value of the α-amino groups in poly peptides is 7.0 - 7.2 at 50°C. The α-amino groups formed are titrated continuously in the pH-stat and the base consumption B (in equivalents) can be related to the number of cleaved peptide bonds n (in equivalents).

The degree of Hydrolysis (DH) is defined as the ratio between the peptide bonds cleaved and the total number of peptide bonds:

1. $$B = \frac{10^{pH-pk}}{1 + 10^{pH-pk}} \cdot n = \alpha \cdot n \text{ equivalents}$$

2. $$DH = \frac{\text{Number of peptide bands cleaved}}{\text{Total number of peptide bonds}}$$

By combining 1. and 2. the DH of the hydrolysate can be calculated:

$$DH = \frac{B}{\alpha \cdot m \cdot n_{tot}} \cdot 100\%$$

where:

B - baseconsumption in equivalents

α - defined from (1)

m - protein (6.25 · N) in kg

n_{tot} - total number of peptide bonds per kg protein

Both the hydrolysis process and the calculation of the results are dealt with in greater detail by Adler Nissen(ref. 6-7).

Experimental Section

Materials

Blood freshly drawn from pigs by means of a hollow knife into plastic containers to which a citrate solution, equalling a trinatrium citrate, concentration of 6g/l blood is added, in order to prevent coagulation. The enzyme used with best results is Alcalase® 0.6 L, a liquid food-grade preparation of Subtilisin Carlsberg. Alcalase® is commercially available from NOVO Industri A/S, Denmark. All other reagents are analytical grade laboratory chemicals.

H 5:4

Methods

Blood is separated into a plasma fraction and a cell fraction by centrifugation. The cell fraction containing about 32-34% protein is diluted 200-300% by adding water, hereby rupturing the cells in order to facilitate enzymatic digestion of their haemoglobine content.

Some solutions are centrifuged prior to hydrolysis in order to remove cell material leaving a purer haemoglobine substrate. Others are treated at pH 11 and the centrifuged after neutralisation, since this treatment has been claimed to accelerate the enzymatic digestion by denaturation of the proteins (Ericksson et al 5).

The protein solution is adjusted with water to the desired substrate concentration S (defined as total protein N x 6.25), whereupon the pH and temperature are adjusted and the enzyme is added. In the following an expression E:S defines the percentage w/w of the ratio of enzyme E relative to substrate S. The reaction is carried out in a 1000 or 3000 ml thermostated pH-stat reaction vessel and monitored by addition of 4 N NaOH, thus titrating the free α-amino groups formed.

Hydrolysis is continued until the desired Degree of Hydrolysis DH (1) is obtained and the reaction is terminated by lowering the pH-value to 4.2 which value is maintained for one hour at the reaction temperature hereby inactivating the enzymes irreversibly.

After centrifugation of the reaction mixture a calculated amount of activated carbon 0.2 - 1% by weight is added to the hydrolysate supernatant which is treated for one hour, whereupon the carbon is removed by filtration. The hydrolysate is then neutralized and freeze-dried. Figure 1 shows a flow sheet of the process.

Gel chromatography characterizing the hydrolysates with respect to molecular distribution is carried out on a K 16/100 column (Pharmacia, Uppsala, Sweden) packed with Sephadex G-25 fine and eluated with a pH 8.2 tris-buffer. The eluation pattern is recorded by measuring the optical density (O.D.) at 280 nm.

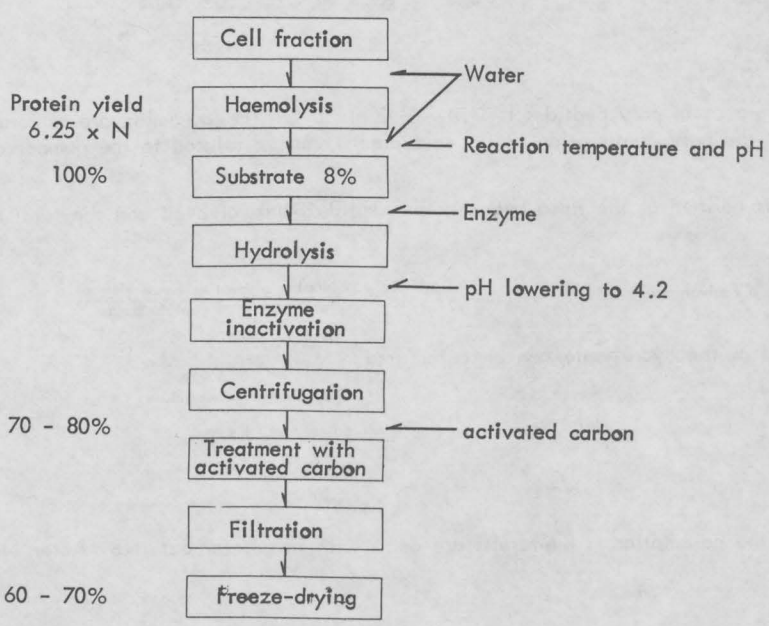
Results

Preliminary experiments show that the enzyme Alcalase® is suitable for hydrolysing the haemoglobine into peptides.

Temperature

Alcalase has maximum activity around 60°C. However, in order to minimize the effect of the enzyme denaturation on the results, a reaction temperature of 55°C is chosen. At this temperature the remaining activity after one hour is still 90% of the starting activity, while at 60°C only 70% activity is left after one hour. All results stated below are obtained at 55°C.

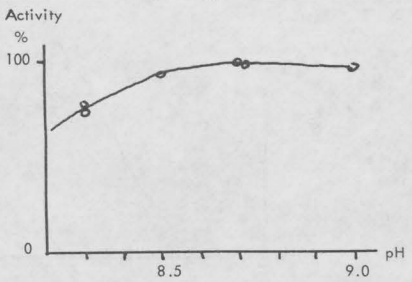
Figure 1



The connection between the activity and the pH-value (in the given system) is analyzed using as a parameter DH after 60 minutes, (Figure 2), during which time the reaction curve is linear, when the conditions are S = 8%, (E:S) = 2%, and temperature = 55°C.

Based on these results the pH-value is chosen to be 8.5, taking into account the better decolourisation effect obtained at lower pH-values as well as minimizing the amounts of base and acid necessary for the adjustment of the pH-value in order to initiate and terminate the reaction. The results stated below are obtained at a pH-value of 8.5.

Figure 2



Substrate concentration S

A substrate concentration variation between 6 and 12% does not affect the reaction speed as long as the (E:S) is kept at a constant level. The substrate concentration is kept at 8-9% in the experiments described below.

Enzyme-substrate ratio (E:S)

Figures 3 and 4 show the relation between the reaction speed and (E:S). As expected, the speed increases by increasing E:S as long as the enzyme is fully saturated with substrate. Thus, the choice of parameters is a compromise between reaction time and enzyme costs. It appears from Figures 3 and 4, that an increase of the E:S ratio results in a drastic increase of the initial activity as well as of the DH-value obtained after one hour.

Figure 3

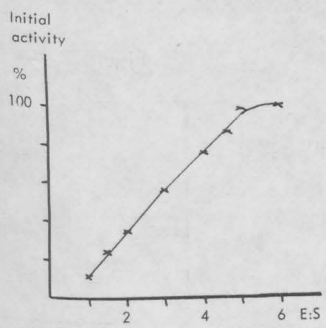
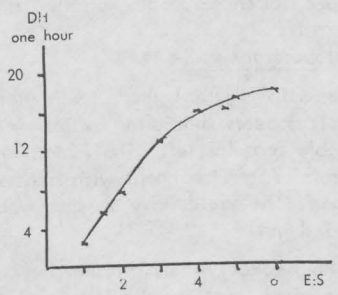


Figure 4



Pretreatment of the substrate

Som authors (5) have pointed out that a predenaturation of the haemoglobine by increasing the pH-value to 11 for one or two hours prior to hydrolyzing should give drastic increases of the reaction speed.

Various combinations of pretreatment at pH 11 have been tried in this reaction system and so far no such effect has been found.

Terminating hydrolysis

Terminating the reaction may be done either by pasteurisation or like here by lowering the pH-value to 4.2 and maintaining this pH for one hour at the reaction temperature. For this purpose an organic acid such as citric acid or an inorganic acid such as hydrochloric acid may be used. Citric acid does improve the subsequent decolourisation carried out with activated carbon. However, when using a weak acid as citric acid, the necessary amount to be added makes up as much as 30-40% of the dry matter in the final neutralized product, mainly as sodium citrates, see Table 1. As opposed hereto, hydrochloric acid may be used in smaller amounts and will give a desirable taste of salt to the product when neutralized with sodium hydroxide and especially in meat products the use of a hydrolysate prepared with hydrochloric acid seems to be desirable.

Table 1

added acid	sodium salt % of hydrolysate dry matter
citric acid	30-40
hydrochloric acid	8-12

Treatment with activated carbon

As mentioned above, this decolourisation treatment was most efficient when using citric acid for enzyme inactivation, whereas hydrochloric acid having other advantages as well, does give acceptable results.

The value of pH during the treatment with activated carbon does have a significant effect on the decolourisation. This may be seen from Figure 5 which is based on colours determined photographically in a hydrolysate solution containing 6% protein (6.25 x N). Treatment at different pH-values has been performed on hydrolysates from the same batch.

Figure 5

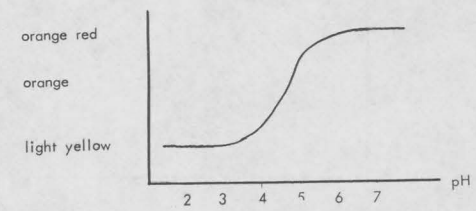
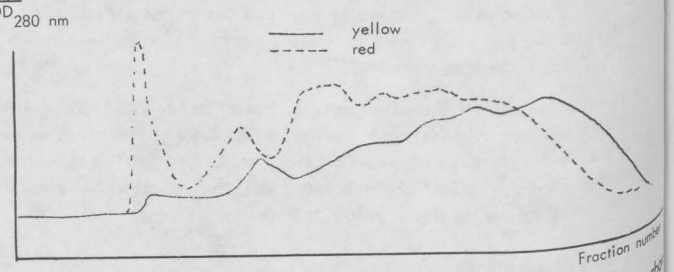


Figure 6



Besides the pH-effect on the decolourisation which is most efficient at pH-values below 4, the amount of activated carbon and the temperature during the carbon treatment of the reaction mixture have a significant influence. As might be expected, decolourisation increases with increasing amounts of carbon such as 1% addition, being a realistic maximum and ½% giving acceptable results.

Decolourisation increases with increasing temperature, and treatment with activated carbon is therefore done at a temperature of 55°C.

Gel chromatography of hydrolysates

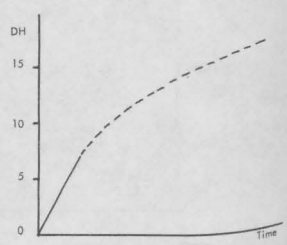
Typical hydrolysate elution patterns from the Sephadex G-25 column is shown in Figure 6. Two graphs of different DH-values are shown. Both solutions are clear and translucent.

Process yield in laboratory scale

On the flow scheme in Figure 1 the protein yields (6.25 x N) at three different steps are shown. It appears that after hydrolysis and enzyme inactivation there are 70-80% soluable proteins left. The remaining precipitate may serve as animal feeding stuff. After treatment with activated carbon a total yield of 60-70% is obtained. The yields may be improved when further process developments are tried out.

Figure 7 shows the typical process relation between DH and time, where the dotted part of the graph contains the DH-range in which decolourisation by carbon treatment is possible.

Figure 7



Discussion

The use of enzymes in the food industry is a field of progress due to the great advantages in this kind of natural modification of proteins, i.e. with respect to functional properties or as here with respect to colour.

The method described in this paper gives a way of processing the red cell fraction into a peptide blend without hydrolyzing completely into amino acids. From the gel chromatographic behaviour of those peptide blends it has been shown that the colour of the hydrolyzed solution is not only related to DH, but also at a given DH to variations in the peptide molecular weight distribution in the solution, and in the cases with peaks indicating the presence of large haemoglobine sub-fragments a bad decolourisation is obtained.

A temperature of 55°C at pH 8.5 with substrate concentrations in the range of 6-12% protein gives satisfactory results with respect to decolourisation when treated with ½-1% activated carbon for 30-60 minutes at a pH-value below 4. In order to obtain satisfactory decolourisation of the product according to this method a DH-range of 7-15% is necessary, (Figure 7).

Hydrolysates prepared according to this method may be used in solutions where a high pH-independent solubility is desirable, even in the presence of high salt concentrations. The described method is being developed further and attempts to characterize the product are being done, especially with respect to application in the manufacture of meat products.

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