<u>A process of discolouration of slaughter house blood : some technical and economical results</u> HOULIER, B.

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# Introduction

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As it has become compulsory in France, since 1983, for slaughter houses to collect blood in satisfactory conditions, the interest in giving a good value to this by-product of the meat industry has been stimulated. Slaughter house blood is a source of valuable animal proteins, quantitatively and qualitatively (RANKEN, 1977) : unfortunately, up to now, slaughter house blood and its components have been insufficiently used in human nutrition in France. The two mains outlets are the traditional manufacturing of black pudding and the processing into blood plasma.

Blood plasma is one of the two products coming from centrifugal separation of hygienically collected blood added with anticoagulant : it contains 20 % of blood proteins which show very good techn<sup>o</sup>cological properties (WISMER-PEDERDEN, 1979). The other separated fraction is the blood corpuscules or blood cells fraction; it contains 80 % of the blood proteins : the main protein of this blood fraction is haemoglobin which becomes quickly oxidised and which would give an undesirable black or dark red colour if used as food additive. In other words, when blood is separated into plasma and blood cells, only 20 % of its proteins are given a high value. Therefore many experiments have recently been carried out in order to disguise or to eliminate the deep-red colour of haemoglobin, while keeping as much as possible the functional properties of these proteins. Discolouration consists in splitting the molecule of haemoglobin into the haem pigment and the globin part, and keeping the latter. The literature describes several process but none of them reached the industrial stage. Previously several process were reported using chemical technology : either hydrogen peroxide (VAN DEN OORD and WESDORD, 1979) or acidified aceton (CORCUFF and al., 1985), or carboxy-methyl cellulose (AUTIO and al., 1984).

Another process, using enzymatic technology was proposed (HALD-CHRISTENSEN, 1978). The evaluation of slaughter house blood collecting and processing equipement (HOULIER, 1985) involved us into the carrying out and monitoring of experiments which were part of a national technological project. This study reports, from recent experimental data, the economic and technical feasability of discolouring the blood cell fraction by a controlled enzymatic hydrolysis.

The objective of this present work is to monitor, from pilot-plant scale trials, the investisment and running costs of such a biotechnological process in connexion with the properties of the final product : the enzymatic dicoloured blood cell (E.D.B.C.) fraction.



Figure 1 : Mass flow-sheet of discolouration process of the blood cell fraction by protease hydrolysis, followed by drying



Figure 2 : Process flow sheet for enzymatic blood cell discolouration and plasma ultrafiltration concentration

# Materials and methods

In a slaughter house (CIBEVIAL, Lyon) producing 25 000 tonnes carcass per year, bovine blood was collected (55 cattle head/hour) under hygienic conditions into a stainless steel suitable trough. A citrate solution (10 g of trinatrium citrate and 1,5 g of sodium chloride per litre of blood) is added in the blood-collecting trough to prevent coagulation. The enzyme used was Alcalase (0,6L, a liquid food-grade preparation of Subtilisin Carlsberg. Alcalase (0) is a commercially available enzyme from NOVO Industry A/S Denmark. All other reagents are analytical grade laboratory chemicals. A volume of 2 500 l of blood is separated into a plasma fraction and a cell fraction by centrifugation.

A volume of 2 500 l of blood is separated into a plasma fraction and a cell fraction by centrifugation. The cell fraction containing 32 % protein is diluted 250 % by adding water, hebery rupturing the cells in order to facilitate enzymatic digestion of their haemoglobine content. The protein solution is adjusted to the desired substrate concentration (8 %) whereupon the pH and temperature are adjusted and the enzyme is added (E/S = 4 %). The reaction is carried out at pH 8,5, at 55°C in a 3 500 Litre vessel. The pH is monitored by additon of 4 N NaOH. Hydroly.sis is continued until the desired degree of hydrol sis (DH = 18 %) is obtained (REGNIER, 1983). The reaction is stopped by lowering the pH to 4,0 which value is maintained from 1/2 hour. After separation of haem from globin protein and peptides by ultrafiltration, a calculated amount of activated carbon is added to the hydrolysate permeate. The hydrolysate is then filtered, concentrated by reverse osmosis and dried by drum-drier or a spray-drier. Figure 1 shows a mass flow-sheet of the process. Figure 2 shows a process flow-sheet (blood-cell enzymatic discolourization and plasma concentration by ultrafiltration).

The energy, water and chemicals consumption have been monitored in order to evaluate the process from the economic point of view. The production costs of enzymatic discoloured blood cell (8 % TS) and concentrated plasma (24 % TS), using ultrafiltration technology have been analysed. Two daily blood processing capacities were taken into account : 2 000 litres and 5 000 litres giving respectively 1 200 litres and 3 000 litres plasma and 800 litres and 2 000 litres blood cell fraction.

### Results and discussion

a) The production costs of the enzymatic discolouration of blood cells with the NOVO endoproteinase are presented in table 1. The plant investisment, which is mainly consisting of stainless steel vessels and ultrafiltration equipment, is only multiplied by 1,6, when the processing capacity goes from 2 000 litres of blood to 5 000 litres of blood. In order to process 5 000 litres of blood (3 000 l of blood cells) the cost of enzymes and chemicals is over 46 % of the total production cost. This explains the relatively small econmy of scale which is achieved if the treated capacity is increased. The process requiring little labour, it can be observed that productivity is increased if the treated capacity increases. The separation between the haem fraction and the globin peptides fraction is executed by ultrafiltration and not , as previously described (HALD-CHRISTENSEN, 1978) by centrifugation. The energy consumption is only 5,5 % of the production cost, which is relatively low. If there is a possibility to use the product as a liquid concentrated form after reverse osmosis, the energy expenses are considerably lowered compared to drying.

Blood volume (1/day)	: 2 000	Relative cost (%)	: : 5 000	Relative cost (%)
Blood cell volume (l/day)	800		2 000	
Capital investment cost (in FF)	indong to i	no La subsit-	There is a	tion cold
. Building	: 107 600 :	reason from	: 145 500	boold-base
. Plant	: 629 000	tral Hoves	:1 033 000	iversetie is
Total (FF)	: 736 600	estand to se	:1 178 500	
Capital depreciation and maintenance cost (FF/day)	:		:	:
. Depreciation allowance : - Buildings	: 38.69 :	2.03	: 52.32	: 1.40
- Plant	: 464.99 :	24.33	: 763.65	: 20,40
. Maintenance	: 84,16 :	4,40	: 161,59	: 4,32
Schurely arter slaughter say have a detrimental tough	:		:	:
Running cost (FF/day)	: cale 126 ha	ar Bedda-men	to belth as	and then :
. Labour	: 252,95 :	: 13,23	: 336,47	: 9,00
. Electricity	:	:	:	:
- Light	: 5,95 :	: 0,32	: 9,47	: 0,25
- Energy	: 83,33 :	4,36	: 207,85	: 5,56
. Enzyme	: 245,22	: 12,83	: 863,05	: 23,06
. Chemicals	: 457,86	: 23,95	: 870,90	: 23,27
. Hot water	: 50,70	: 2,65	: 126,75	: 3,38
. Membrane remplacement	: 34,92	: 1,83	: 87,44	: 2,33
. Cleaning costs	: 192,57	: 10,07	: 262,99	: 7,03
Total daily running cost (FF/day)	:1 323,50	69,24	:2 764,92	73,88
Total : Running, depreciation and maintenance (FF/day)	:1 911.34	100.00	:3 742.48	: 100.00
Cost in FF/kg of treated blood cell	: 2,390	: _	: 1,871	: _
Weight of EDBC (8 % TS) obtained (kg)	:2 100		:5 250	: -
Cost (FE/kg EDBC nowdon of this process	: 10 55	PERSONAL PROPERTY	: 8 29	
Production cost of 1kg of EDBC (8 % TS)	: 0,910	an service ( a d	: 0 708	
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Table 1 : Cost of enzymatic discolouration of blood (in FF)

Blood volume (1/day)	: : 2 000	Relative cost (%)	: : 5 000	Relative cost (%)
Plasma volume (1/day)	: 1 200	Cart Lines La	: 3 000	
Capital investment cost (in FF)	:	:	:	:
. Buiding	: 107 600	M JASAAVSS	: 145 500	10000203-3
· Plant	: 629 000	the <u>Conc</u>	:1033 000	:
Total (FF)	: 734 000		::	:
Capital depreciation and maintenance cost (FF/day)	:		:	:
. Depreciation allowance : - Buildings	: 19.41	2.81	: 26.25	: 2.17
- Plant	: 233.20	: 33.77	: 382.98	: 31.64
. Maintenance	: 42,22	: 6,11	: 81,05	: 6,70
Running	Suchear fo		:	:
cost (FF/day)	:	:	:	:
· Labour	: 96,42	: 13,97	: 159,78	: 13,20
· Liectricity	: 2.00	. 0.4.2	:	:
- Light	: 2,99	0,43	: 4,75	: 0,39
- Ellergy	· 41,79	. 6,05	: 104,24	: 0,61
Steam	• 12 00	. 1,74	. 130,22	· 11,51
. Membrane remplacement	: 17.46	2 53	· 43 72	. 2,40
· Cleaning costs	: 170,23	: 24,65	: 240,65	: 19,88
Total data	:	:	:	:
daily running cost (FF/day)	: 395,70	57,31	: 720,06	: 59,50
<pre>'otal : running, depreciation and maintenance (FF/day)</pre>	: 690,53	100,00	: 1210,34	: 100,00
Weich (FF/kg of plasma)	: 0,575		: 0,403	: -
Weight of concentrated plasma (24 % MS) (kg)	: 400		: 1000	: -
Cost (For plasma powder (93 % TS) (kg)	:	Se seele s	:	:
CIFF/kg of concentrated plasma	: 1,73	Aust To ba	: 1,21	: -

Table 2 : Cost of ultrafiltration concentration of plasma (in FF)

Considering a plant processing a daily 5 tonnes of blood, the production cost of 1 kg of discolourized globin (8 % dry matter) is about 0,70 FF, that is to say 8,30 F for 1 kg of discolourized globin powder (93 % TS).

b) As for the ultrafiltration concentration of plasma, table 2 shows the capital investisment cost and the production costs. There is a reduction of production cost from 0,575 FF/kg plasma to 0,403 FF/kg plasma if the treated blood volume increases from 2 000 litres to 5 000 litres. In other words, an economy of scale can be observed : the capital investisment cost per processed litre is relatively lower (64,2%) if a daily volume of 3 000 litres of plasma is treated than if 1 200 litres of plasma is treated. The capital investisment cost and the equipment used is the same as for the enzymatic discolouration of blood : both operations should be done successively in the same plant. The envisaged plant must be designed in such a way that plasma ultrafiltration comes as soon as this one is separated by centrifugation from the blood cells or corpuscules : this process gives more efficient results if it is performed at a temperature of 30°C. The ultrafiltration separation of the hydrolysate from the haem can be postponed, if necessary without any inconvenient, because the product is stabilised.

c) Plasma can then be dried by a drum-drier or a spray-drier, but the best functional properties have been obtained if it is dried by a spray-drier (DELAITRE and LORIENT, 1985): mostly emulsifying properties are much better.

d) The functional properties of enzymatically discolourized blood cells have been studied (DELAITRE and al., 1984). The solubility is complete for all pH and all ionic concentration. The foaming capacity is good but the stability of the foam is poor. The emulsifying capacity of the hydrolysed globin is excellent but a major drawback is the poor stability of the emulsion.

However, a mixture of 2/3 plasma - 1/3 globin hydrolysate shows good emulsifying properties (superior to caseinate) and greater foaming capacity than the egg white. It is a white to yellow coloured product with interesting functional properties. With a selling value of about 21 FF (\$ 3,00) it should give the best value to 1 litre slaughter house blood; in this way, all the blood proteins are given a good valorization.

## Conclusion

The process of enzymatic hydrolysis of haemoglobin used in order to discolourate the blood cell fraction, associated to the process of plasma concentration by ultrafiltration, have been experimented at CIBEVIAL (Lyon slaughter house complex) in satisfactory conditions. This process is compatible with the good work of such a large slaughter house. Although the functional properties of the enzymatically discoloured blood cell fraction, consisting mainly of peptides, are rather poor, they can largely be improved by mixing with plasma. Even for a slaughter house processing 25 000 tonnes of carcass per year, the economic interest

of this blood treatment process remains uncertain. It will depend on the selling price of the mixture discolourized blood cell-plasma. In any case, in order to make this process plant profitable, the cost of the proteolytic enzyme should become lower. Besides, the volume of treated volume should be the result of the collection from several slaughter houses.

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